THE UNIVERSITY OF ALBERTA

EFFECTS OF LIVE STEAM TREATMENT OF CANOLA SEED

by

© Slobodan Levi

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

IN

Food Chemistry

Department of Food Science

EDMONTON, ALBERTA

Fall 1986

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ISBN 0-315-32326-4

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Slobodan Levi

TITLE OF THESIS

EFFECTS OF LIVE STEAM TREATMENT OF CANOLA SEED

DEGREE FOR WHICH THESIS WAS PRESENTED **Master of Science**

YEAR THIS DEGREE GRANTED **Fall 1986**

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled **EFFECTS OF LIVE STEAM TREATMENT OF CANOLA SEED** submitted by **Slobodan Levi** in partial fulfilment of the requirements for the degree of **Master of Science** in **Food Chemistry**.

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Supervisor

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Date....OCT. 14, 1986

DEDICATION

To Michele

ABSTRACT

Effects of wet steam treatment of whole and crushed canola oilseeds were studied with respect to oil yield, composition and fatty acid pattern of the oil fractions, phosphorus, sulfur, chlorophyll and carotenoid concentrations in the oil, and volatile, and nonvolatile (hydroxyl group-containing) glucosinolate breakdown products in the meal.

While the oil yield and composition, as well as the fatty acid patterns of lipid fractions in the oil did not differ significantly between steam- and dry heated samples, increases in phosphorus, sulfur, chlorophyll and carotenoid contents in both pressed and extracted oils from steam-treated seed were highly significant. The concentrations of volatile and nonvolatile isothiocyanates were 0.22 mg/g and 0.14 mg/g, respectively, in canola meal from steam-treated seed, while the corresponding concentrations for dry heat treatment were 1.64 mg/g and 1.42 mg/g, respectivly. The concentration of intact glucosinolates, however, was significantly higher in meals from steam-treated seeds as compared to the samples from dry heated seeds.

Lipase, phospholipase and lipoxygenase activities were not evident in crushed seed held up to 30 min at room temperature since the composition of lipid fractions and their fatty acid patterns remained unchanged.

As found by transmission electron microscopy, the size distribution of lipid bodies within cells of outer and inner

cotyledons and central meristem was rather uniform: over 90% of lipid bodies were between 0.3 and 0.7 µm in diameter, which was the size range of 86-100% of globoids embedded in aleurone grains. The size of the latter species was most variable, ranging between 1 µm and 7 µm in some parts of the inner cotyledon.

The assay of bleaching efficiency of Na-activated clay and naturally-active clay proved the former to be superior to the latter in a concentration range of 0.03 to 2%.

ACKNOWLEDGEMENTS

I would like to sincerely thank Dr. Dimitri Hadziyev, my supervisor, for his advice and encouragement throughout the course of this study.

I wish to express my gratitude to Alberta Food Products (AFP) and to the Department of Food Science for providing financial aid, without which the completion of this project would not have been possible.

Special thanks to Mr. Petros Loutas for his useful advice on canola biochemistry and to Mrs. Eva Hofbauer for providing information on methodology in lipid analysis.

Many thanks to Mr. Brent Gillespie for his assistance in gas chromatographic analysis of fatty acids, as well as in spectrophotometric analysis of glucosinolates.

The assistance of Mr. Len Steele in the preparation of the manuscript is gratefully acknowledged.

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LIST OF ABBREVIATIONS

3-BITC	3-Butenylisothiocyanate
FAME	Fatty Acid Methyl Ester(s)
FFA	Free Fatty Acids
GL	Glycolipids
GLC	Gas-Liquid Chromatography
NL	Neutral Lipids
P	Phosphorus
PC	Phosphatidyl Choline
PE	Phosphatidyl Ethanolamine
PI	Phosphatidyl Inositol
PL	Phospholipids
ppm	Parts Per Million
S	Sulfur
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
TG	Triglycerides
TLC	Thin-Layer Chromatography
5-VOT	5-Vinyloxazolidinethione

1. INTRODUCTION

In the industrial processing of canola oilseeds, dry heat is applied in the cooking step. This approach had first been used in the processing of rapeseed cultivars high in erucic acid and in glucosinolates. In the crushing step of rapeseed processing, endogenous myrosinase (thioglucoside glucohydrolase) enzyme is brought into contact with glucosinolates, resulting in rapid hydrolysis if the seed moisture is >8.5% and toxic isothiocyanates, nitriles and elemental sulfur are produced, thus seriously degrading the quality of the oil and meal.

The introduction of commercial "double zero" cultivars (low both in erucic acid and in glucosinolates) in 1974 was followed by a number of new cultivars of similar quality. These were designated in Canada in 1979 as "canola" (Canadian Oil). Despite the rapid take-over of the market by canola cultivars, the design of stack cookers remained adjusted for the processing of rapeseed high in glucosinolates, i.e. for the dry heat process.

The idea of steam cooking of oilseeds is not new, but its application in processing of rapeseed had been limited for the previously discussed possibility of glucosinolate hydrolysis. The principal object of heat treatment is to coagulate the proteins in the walls of lipid-containing cells and thus make the walls permeable to the flow of oil. The flow of oil from the oil-bearing material is also assisted by the lowered viscosity of the oil at elevated temperatures. Water must be present for protein coagulation to take place. Anhydrous proteins do not readily coagulate or exhibit other evidence of heat denaturation. In some cases, water also assists in the displacement of the oil from the surfaces of solid materials due to the superior physicochemical affinity of water for these materials.

In the case of high glucosinolate rapeseed cultivars, moisture content of the seed has to be kept between 6 and 8% because at moisture contents <6% myrosinase resists heat inactivation, while moisture contents >8.5% promote rapid hydrolysis of glucosinolates upon seed crushing.

Myrosinase might be active at the initial stages of treatment, but since glucosinolates in canola are low, their partial hydrolysis by the enzyme would not present a problem. More importantly, in the presence of increased moisture content, heat coagulation of protein, including inactivation of enzymes, would take place and an increase in oil yield due to the increased extractability of neutral lipids (triacylglycerols) could be expected. However, steam treatment could also cause an increased concentration of polar lipids (phosphatides) and pigments, both of which are complexed with the proteins, in prepressed/extracted oils.

The design of stack cookers in most crushing plants in Western Canada consists of 5-8 closed, cylindrical kettles which are jacketed for the indirect heating with steam. A conversion from indirect heating to direct steam or combined direct-indirect steam treatment would require only minor modifications in the design. Steam could be injected directly through spray jets in the top kettle(s) and removed through exhaust pipes in the lower kettles. Since the moisture content of the cooked seed must be between 4 and 8% to ensure adequate handling properties and the efficiency of prepressing/solvent extraction, the combination of direct and indirect steam treatment appeared to be the basis for further investigation.

In the first part of this project, the effects of endogenous lipase, phospholipase and lipoxygenase were studied in seeds after crushing, with holding times of 0-30 min at room temperature. Subsequently, the composition of lipid fractions and their respective fatty acid patterns were analysed.

In the second part of this project, a comparative analysis of steam and dry heat treatments of whole and crushed canola seeds was carried out to determine the total oil yield, composition of oil fractions and their fatty acid patterns, and concentrations of phosphorus, sulfur, chlorophyll and carotenoids in the oil. Finally, the levels of intact and autolysed glucosinolates in residual meal were investigated.

Scanning and transmission electron microscopy were applied to obtain additional understanding of the seed structure, the anatomy of individual cells and the morphology of lipid and aleurone grains in the cells.

2. LITERATURE REVIEW

2.1 General Aspects

Rapeseed is one of the major sources of edible vegetable oils. It ranks fourth in the world production of vegetable oils, being in front of such sources as peanut and cottonseed oils. High oil content of the seeds and protein-rich meal with an amino acid pattern comparable to that of soybean meal (Clandinin, 1981), coupled with wide adaptability to different climatic regions of the world, make this crop attractive to many developed and developing countries.

Canada has achieved a distinguished role in rapeseed production for at least two reasons: it is the largest producer of rapeseed in the world, and it pioneered the commercial production of canola cultivars low in both erucic acid ($C_{22:1}$) and in glucosinolate content. This significantly improved the quality of edible rapeseed oil and of meal used as animal feed. By 1983, practically all Canadian cultivars were of canola quality.

Over the past five years, production of canola crushed seed, oil and meal have increased by 22%, 19% and 25%, respectively (Table 2.1). During this time the soybean industry recorded a stagnating period.

With approximately 35% of total Canadian production in 1985, Alberta is the country's second largest producer after Saskatchewan (Statistics Canada).

Table 2.1 Oilseed crushing in Canada'.

	Soybean (tonnes)	Canola (tonnes)
Quantity Crushed		
1980-81	929,690	1,003,281
1981-82	961,905	945,354
1982-83	1,043,224	904,096
1983-84	937,205	1,159,322
1984-85	928,275	1,290,442
Oil Produced		
1980-81	158,931	418,159
1981-82	164,297	382,127
1982-83	179,316	366,181
1983-84	166,256	456,441
1984-85	169,789	514,446
Meal Produced		
1980-81	731,702	573,572
1981-82	757,470	551,066
1982-83	832,574	521,712
1983-84	735,953	688,058
1984-85	721,520	767,828

' Statistics Canada, 1985. Cat. No. 22-007.

Summer forms of rape (*B. napus*) and of turnip rape (*B. campestris*) predominate in Canada. Sowing takes place in late April and early May and the crop is harvested in September, resulting in a growing season of about 105 days.

Regent, Altex, Westar (*B. napus*), Candle and Tobin (*B. campestris*), all "double zero" cultivars, are among the most commonly grown cultivars in Canada.

2.2 Chemical Composition of Canola Seeds

2.2.1 Gross composition

Conversion to "double zero" cultivars affected primarily total fatty acid pattern of rapeseed lipids and the glucosinolate content of the meal (Downey, 1983). However, the absolute content and composition of such major constituents as lipids and proteins remained relatively unchanged. The composition of rapeseed is outlined in Table 2.2.

Moisture content of naturally dry rapeseed ranges from 6 to 8% (Persmark, 1972). This moisture content is considered to be relatively low and was attributed to the high oil content in rapeseed as compared with, for example, soybeans.

Appelquist (1972) classified major rapeseed components into lipids, proteins, carbohydrates and glucosinolates. The lipid content of mature rapeseed, as determinned by extraction with nonpolar hydrocarbon solvent, varies over a

Table 2.2 Major chemical constituents of rape (*Brassica napus*) and turnip rape (*Brassica campestris*)[1].

		B. napus			*B. campestris*	
		Westar	Regent	Andor	Candle	Tobin
Oil[2]	1982	43.8	42.1	43.4	42.2	42.9
	1983	43.4	43.2	42.9	41.4	41.7
	1984	42.4	42.1	42.2	41.0	41.2
Protein[3]	1982	44.5	47.3	46.4	43.0	42.6
	1983	45.4	47.3	47.0	42.6	42.2
	1984	45.6	47.0	46.2	42.5	42.8
Fiber[2]	1982	10.6	10.2	10.2	7.7	8.1
	1983	10.4	10.4	10.1	7.9	8.0
	1984	10.6	10.4	10.4	8.1	8.1

[1] Agriculture Canada, Saskatoon, annual report.

[2] Moisture-free basis.

[3] Moisture-free, oil-free basis.

wide range depending on cultivar, growing conditions, etc. (Schuster, 1967).

Analysis of winter and summer rapes gave 42-50% and 37-47% oil, respectively, while 40-48% was found in winter turnip rape and 36-46% in summer turnip rape (Sallans, 1964).

Protein content is usually reported on a lipid-free rapeseed meal basis, and, being subject to interspecies differences, varies between 33-47.9% (Flax and Rapeseed, Can. Crop Bull., 1969).

Rapeseed carbohydrates are present mainly as constituents of cell walls in cotyledons and in the fibrous seed coat. The main polysaccharide constituents are cellulose, hemicellulose and pectic substances, accounting in total for approximately 15% of defatted rapeseed meal (Sosulski and Bakal, 1969). This is high compared to other oilseeds and is related to the small size of the seed (1-1.5 mm) with a large proportion of seed coat. Some cultivars (Candle, Tobin) have lower fibre content (10-11%), are lighter in color and have higher content of oil in seed (42.2%, on a dry matter basis) and of protein in defatted, moisture-free meal (42.2%) (Downey, 1983). These cultivars are often referred to as "triple low".

Since the introduction of "double zero" cultivars, glucosinolates can hardly be called a major component of rapeseed. Many researchers are, however, still attracted to this field since some nonvolatile hydrolysis products may have adverse effects on nutritional quality of the meal, even when present in minute concentrations (McGregor, 1978).

2.2.2 Lipids

2.2.2.1 Neutral lipids

About 95-96% of total canola lipids are represented by neutral lipids (Zadernowski *et al.*, 1978). The major component of this class is triacylglycerols (≅96.5%) followed by diacylglycerols (1.25%), sterol esters (1.15%), sterols (0.63%) and free fatty acids (0.5%).

McKillican (1966) found that neutral lipid composition varies significantly with the maturity of the seeds. While neutral lipids from 10 DAF (days after fertilization) rapeseed samples contained 23% triacylglycerols, 12.3% mono- and diacylglycerols, 15.8% free fatty acids and 9.7% free sterols, the composition of these constituents in mature seeds changed to 94.3%, 1.7%, 0.1%, and 1.1%, respectively.

As a result of breeding towards low erucic acid cultivars, C_{18} fatty acids became predominant in triacylglycerols with $C_{18:1}$ accounting for ≅60% (Table 2.3). C_{54} is the predominant triacylglycerol type in low erucic acid rapeseed (LEAR) oils (combination of 3 x C_{18} fatty acids). Among high erucic acid rapeseed (HEAR) oils, dominant triacylglycerol constituent was C_{62} (from 2 x C_{22} + 1 x C_{18}) (Ackman, 1983). Jaky and Kurnick (1981) suggested that in triacylglycerols of LEAR oils, reduced frequency of $C_{18:2}$ n-6 at C-2 (54%), as compared with 95% in HEAR oils, can be

Table 2.3 Composition (%) of major fatty acids in oils from high erucic acid rapeseed (HEAR), low erucic acid rapeseed (LEAR), sunflower and soybean.

Fatty acids	Plant seed				
	HEAR	LEAR	Sun-flower	Soy-bean	White lupin
16:0	4	3	6	10	7
18:0	1	1	4	4	3
18:1	15	60	18	24	55
18:2 *n*-6	14	20	68	51	15
18:3 *n*-3	9	13	<1	10	9
20:0	1	1	1	1	1
20:1	10	1	--	--	5
22:0	<1	<1	1	<1	3
22:1	45	<1	--	--	2

(taken from Ackman, 1983)

explained by the increased proportion of $C_{18:2}$ n-6 in LEAR oils (≅20% as compared with 14% in HEAR oils) and its redistribution at positions 1 and 3.

HPLC methods have been successfully applied in triacylglycerol analysis. Peterson *et al.* (1981) achieved complete separation of lobra oil (Swedish rapeseed cultivar low in $C_{22:1}$) triacylglycerols based on equivalent carbon number (ECN) using a reverse phase HPLC system with a 100 cm Nucleosil 5 C_{18} column and acetonitrile/acetone, (60:40, v/v) as mobile phase.

Earlier methods for triacylglycerol analysis usually employed two-step techniques such as Ag-TLC/CN-GLC or Ag-TLC/RPHPLC. Reverse phase C_{18} columns have been, however, successfully applied in one-step separations. El-Hamdy and Perkins (1981) accomplished separation of triacylglycerol critical pairs (those having the same ECN), including cis-trans geometrical isomers, using a 25 cm column packed commercially with 5 µm silica bonded with octadecyl stationary phase and a mobile phase of acetone/acetonitrile (63.6:36.4, v/v) as a mobile phase. Plattner (1981) reported an improved method for separation of tripalmitin from triolein and palmitoolein using an m-Bondapak C_{18} column with 0.2 N silver nitrate present in the mobile phase.

Free fatty acid (FFA) content in LEAR oils was found to be 0.5% (Zadernowski and Sosulski, 1981). The same authors found the composition of FFA to be different from that of triacylglycerols. Palmitic acid ($C_{16:0}$) was 5.2% in the triacylglycerol fraction, while it was 19.7% in the FFA fraction. Also, proportions of linoleic (7.8%) and linolenic (4.8%) acids in FFA were found to be significantly lower than in triacylglycerols (20.4% of $C_{18:2}$ and 10.8% of $C_{18:3}$).

Free sterols are a minor component of canola lipids. Kovach *et al.* (1978) reported 0.45-0.975% of total sterols in 14 samples of crude and refined LEAR oils. β-sitosterol was the dominant component, ranging from 47.6 to 59.8%, followed by campesterol, 31.5-37.6%, and brassicasterol, 6.4-11.5%. The latter component is a distinct constituent of rapeseed oil, a fact which is often utilized to identify rapeseed oil in oil mixtures (Spencer *et al.*, 1976). It was suggested by Seher (1976) that TLC should not be applied for sterol analysis in rapeseed oil since campesterol oxidation artifacts might mimic cholesterol, leading to erroneous conclusion about the content of the latter in the oils.

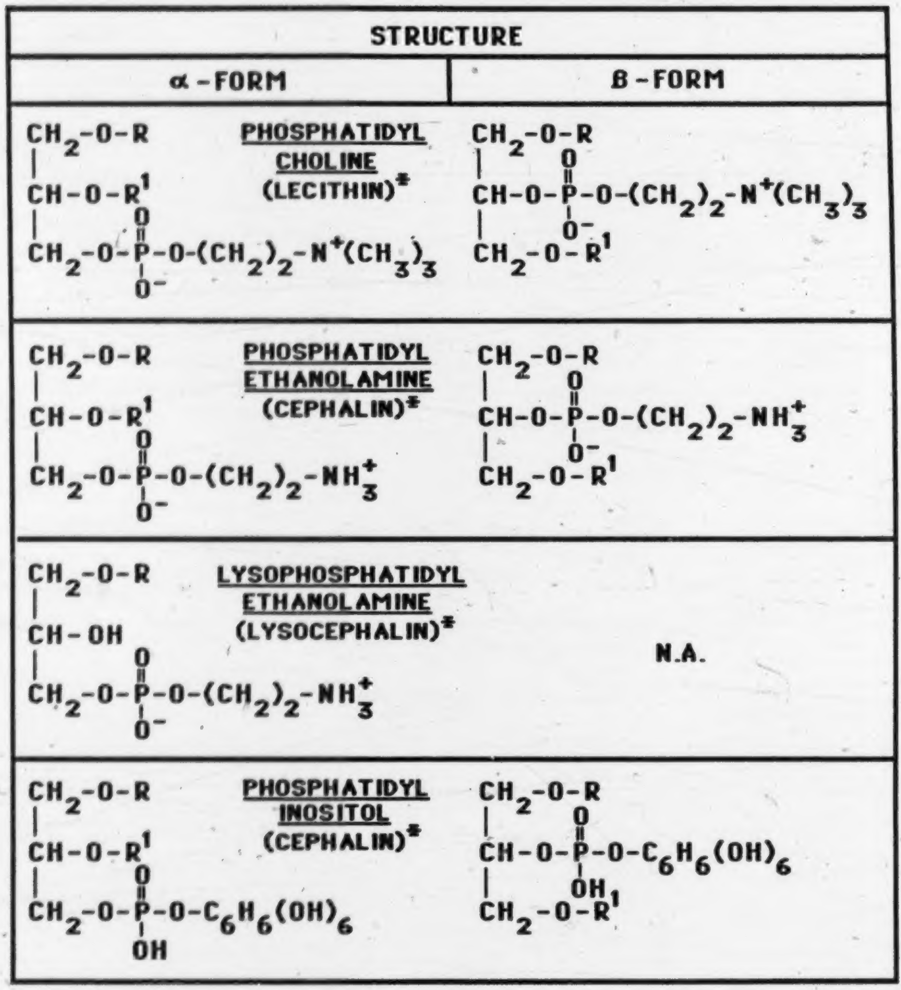
2.2.2.2 Polar lipids

Phospholipids

Phospholipids (Table 2.4) are essential components of cytoplasmic membranes (plasmalemma, tonoplasts) of vegetative and reproductive tissues. Due to their amphiphilic nature, they have an important role in the transport of uncharged and charged molecules, in control of enzyme activities, and in biosynthesis of triacylglycerols (Cherry *et al.*, 1981).

The phospholipid bilayer in cell membranes is bound

Table 2.4 Chemical structures of major hydratable and non-hydratable phospholipids in rapeseed.



STRUCTURE	
α-FORM	β-FORM
CH_2-O-R $CH-O-R^1$ $CH_2-O-\underset{O^-}{\overset{O}{\overset{\|}{P}}}-O-(CH_2)_2-N^+(CH_3)_3$ **PHOSPHATIDYL CHOLINE** **(LECITHIN)***	CH_2-O-R $CH-O-\underset{O^-}{\overset{O}{\overset{\|}{P}}}-O-(CH_2)_2-N^+(CH_3)_3$ CH_2-O-R^1
CH_2-O-R $CH-O-R^1$ $CH_2-O-\underset{O^-}{\overset{O}{\overset{\|}{P}}}-O-(CH_2)_2-NH_3^+$ **PHOSPHATIDYL ETHANOLAMINE** **(CEPHALIN)***	CH_2-O-R $CH-O-\underset{O^-}{\overset{O}{\overset{\|}{P}}}-O-(CH_2)_2-NH_3^+$ CH_2-O-R^1
CH_2-O-R $CH-OH$ $CH_2-O-\underset{O^-}{\overset{O}{\overset{\|}{P}}}-O-(CH_2)_2-NH_3^+$ **LYSOPHOSPHATIDYL ETHANOLAMINE** **(LYSOCEPHALIN)***	N.A.
CH_2-O-R $CH-O-R^1$ $CH_2-O-\underset{OH}{\overset{O}{\overset{\|}{P}}}-O-C_6H_6(OH)_6$ **PHOSPHATIDYL INOSITOL** **(CEPHALIN)***	CH_2-O-R $CH-O-\underset{OH}{\overset{O}{\overset{\|}{P}}}-O-C_6H_6(OH)_6$ CH_2-O-R^1

* "Trivial" name given in parentheses.

to protein through its outer (hydrophobic) side. Zahler and Wibel (1970) reported that protein occupied only one-half of the external layers, the other part being occupied mainly by water.

The interaction between phospholipids and proteins in food systems is determined by the status of the proteins. Kinsella (1979) reported that soybean proteins in their native form had not interacted well with phospholipids. However, dissociated proteins (polypeptides) formed lipoprotein complexes.

Canola phospholipid content decreases with seed maturation. A study by McKillican (1966) showed that the content of polar lipids (glyco- and phospholipids) decreased from 32.2% in 10 DAF zero-erucic rapeseed to 1.8% in mature seeds.

Sosulski (1981) separated total canola lipids on an activated SiO_2 column into neutral (NL), glyco- (GL) and phospholipids (PL), and found the latter component to be 3.3% of total lipids. He analyzed by TLC/GLC the composition of canola phospholipids and the fatty acid pattern in its constituents. The major components were phosphatidylcholine (PC), phosphatidylinositol (PI) and phosphatidylethanolamine (PE), accounting for 48.1%, 19.5% and 8.9%, respectively, with approx. 25% of unidentified phospholipids. Oleic and linoleic acids were the principal acids in all three fractions: 55.8% and 30.9% in PC, 33.6% and 38.1% in PI, 47.7% and 27.3% in PE, respectively.

Chapman and Robertson (1977) found that phospholipid content of soybean oil increased with storage time (0-23 days) at higher temperature (35°C) and relative humidity (85%). The authors concluded that this increase could not have been attributed to mold growth. Sessa *et al.* (1974) found that soybean PC oxidized in an aqueous suspension developed bitter taste. The bitter taste in rapeseed phospholipids can be due to hydrolytic cleavage and formation of melanophosphatides during oil extraction and refining processes (Newiadomski, 1970). The same authors fractionated commercial and benzine (petroleum ether) extracted phospholipids into lecithin (PC) and cephalin (PE) by liquid-liquid and solid-liquid extraction with 95% ethanol and methanol, respectively. TLC separation revealed that PC accounted for 48.8% of the lecithin fraction while lyso-PE, 47.7%, was the major component of the cephalin. This might be of interest in the processing industry since cephalin forms water-in-oil-type (W/O) emulsions, whereas lecithin is used for oil-in-water (O/W) emulsions.

The type of emulsion produced and its stability depend largely on the relative ability of the two phases to wet the solid particles. The phase that preferentially wets the solid particles tends to become the continuous phase. If the interfacial tension between solid and oil is greater than that between solid and water, then the major portion of solid particles resides in the water phase, thus favoring an O/W emulsion. The converse takes place if the interfacial tension between solid and water is greater than that between solid and oil. Consequently, if solid particles remain exclusively in either of the two phases, they have no stabilizing effect. The most stable emulsion is formed when the solid particle surface is equally distributed between liquid and solid phases. The type of emulsion produced can be predicted on the basis of the relative hydrophilic-lipophilic properties of the emulsifier. According to the hydrophilic-lipophilic balance (HLB) concept, each surface-active agent can be assigned a numerical value representing its hydrophilic-lipophilic balance.

Phospholipids such as PC, PE and PI are hydratable since they contain polar groups (choline, ethanolamine and inositol) and can be removed by mixing the crude oil with water (2%) and subsequent centrifugation. However, there is a considerable amount of phospholipids which are not hydratable. These are mainly undissociated phosphatidic and lysophosphatidic acids and Mg/Ca phosphatides (Hvolby, 1970).

Phospholipids can exist in α- and β-form (Figure 2.1). The α-form has the esterified phosphoric radical bound to C-3 (or C-1), while in the β-form the radical is bound to C-2. The β-form is considered nonhydratable (Carr, 1978) and cannot be removed from the oil by a water-degumming process. No data were found on the exact

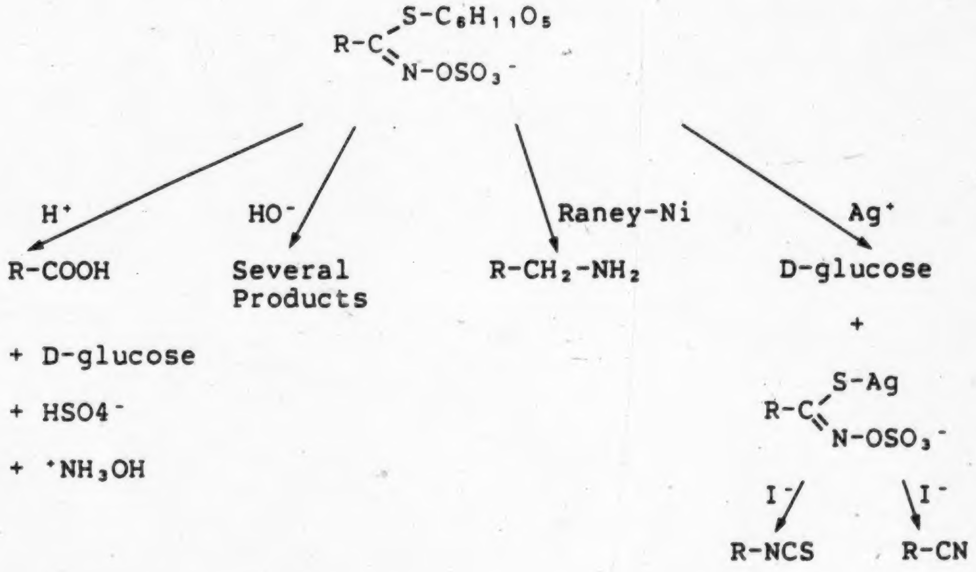

R-C S-C6H11O5 N-OSO3-
H+
HO-
Raney-Ni
Ag+
R-COOH
Several Products
R-CH2-NH2
D-glucose
+
+ D-glucose
+ HSO4-
+ +NH3OH
R-C S-Ag N-OSO3-
I-
I-
R-NCS
R-CN

Figure 2.1 Nonenzymatic degradation of glucosinolates.

concentration of β-phospholipids, undissociated phosphatidic acid, and its Mg- and Ca-salts in rapeseed/canola oils. However, Persmark (1972) reported that the total amount of nonhydratable phospholipids in rapeseed oil can be as high as 50%.

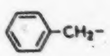
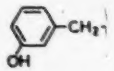
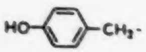
2.2.3 Glucosinolates

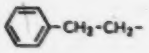
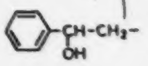
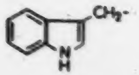
2.2.3.1 Occurrence and chemistry

Glucosinolates are often found in the literature under such names as thioglucosides or mustard oil glucosides (Appelquist, 1972). These compounds are commonly referred as toxic components of rapeseed meal. The exact cytological distribution of glucosinolates in rapeseed is still subject to investigation (Von Hofsten, 1970). The term "glucosinolates" encompasses over 70 individual compounds varying only in the character of the side chain (R). The chemical structures and major types of glucosinolates are shown in Table 2.5.

Acid-catalyzed hydrolysis of a glucosinolate may yield hydroxylamine, sulphate, a sugar moiety and a carboxylic acid (Figure 2.2). In basic solutions, glucosinolates are transformed into several products. Compounds containing activated hydrogen at C-1, i.e. allyl- and benzyl glucosinolates, form the corresponding α-amino acids and thioglucose. Some R-groups, e.g. phenols, are also unstable in basic solutions (Olsen *et al.*, 1980). Gronowitz *et al.* (1978) found that 3-butenylglucosinolate (gluconapin) and

Table 2.5 Chemical structures of glucosinolates.

Compound No.	Group R	k'	Glucosinolate name	Trivial name of salt
1	CH_3-	3.3	methylglucosinolate	glucocapparin
2	CH_2=CH-CH_2-	4.2	allylglucosinolate	sinigrin
3	CH_2=CH-$(CH_2)_2$-	5.0	but-3-enylglucosinolate	gluconapin
4	CH_2=CH-$(CH_2)_3$-	5.5	pent-4-enylglucosinolate	glucobrassicanapin
5	CH_2=CH-CH(OH)-CH_2-	3.4	2-hydroxybut-3-enylglucosinolate	progoitrin
6	CH_2=CH-CH_2-CH(OH)-CH_2-	3.7	3-hydroxypent-4-enylglucosinolate	napoleiferin
7	CH_3-SO-$(CH_2)_3$-	2.1	3-methylsulfinylpropylglucosinolate	glucoiberin
8	CH_3-SO-$(CH_2)_4$-	---	4-methylsulfinylbutylglucosinolate	glucoraphinin
9	CH_3-SO-$(CH_2)_5$-	---	5-methylsulfinylpentylglucosinolate	glucoalyssin
10	CH_3-SO_2-$(CH_2)_3$-	2.9	3-methylsulfonylpropylglucosinolate	glucocheirolin
11		6.7	benzylglucosinolate	glucotropaeolin
12		6.4	m-hydroxybenzylglucosinolate	glucolepgramin
13		5.0	p-hydroxybenzylglucosinolate	sinalbin

14		9.8	phenethylglucosinolate	gluconasturtiin
15		6.2	2-hydroxy-2-phenylethylglucosinolate	glucobarbarin
16		9.8	indol-s-ylmethylglucosinolate	glucobrassicin

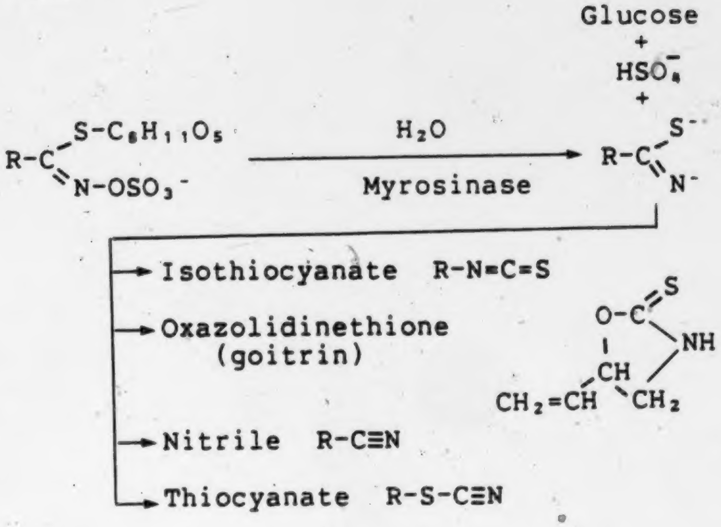

Glucose
+
HSO_4^-
+
$R-C(S-C_6H_{11}O_5)=N-OSO_3^-$
H_2O
Myrosinase
$R-C(S^-)=N^-$
Isothiocyanate R-N=C=S
Oxazolidinethione
(goitrin)
O-C
S
NH
CH
CH_2=CH
CH_2
Nitrile R-C≡N
Thiocyanate R-S-C≡N

Figure 2.2 Enzymatic hydrolysis of glucosinolates.

2-hydroxy-3-butenylglucosinolate (progoitrin) were unstable in both acidic and basic solutions. The greatest stability was found in neutral solutions but it was evident that glucosinolates were not very stable in aqueous solutions. Solutions containing mercury or silver ions resulted in the formation of D-glucose and the metal derivative of the aglucone (Figure 2.2). The metal derivative can be further degraded under controlled conditions to the corresponding nitrile or isothiocyanate, depending on pH of the solution.

Glucosinolates can be also reduced to primary amines using Raney-Nickel catalyses in aqueous solution at room temperature (Ettlinger, 1956). The position of the amino group is at the carbon atom corresponding to C-0 in the glucosinolates; thus, these amines are different from the amines proposed as catabolic products of glucosinolates (Olsen *et al.*, 1980; Sorensen, 1970; Dalgaard, 1977). The amino group in these compounds is at the carbon atom corresponding to C-1 in glucosinolates.

Enzymatic hydrolysis, however, catalyzed by a group of enzymes with the common name "myrosinase" (thioglucoside glucohydrolase) has a different pattern (Figure 2.2). In this case, glucosinolates decompose to D-glucose and aglucone (thiohydroxamate-0-sulfonate) followed by molecular rearrangement of the latter and loss of sulfate to produce an isothiocyanate as a stable end-product (Kjaer, 1970). Another possible pathway in enzymatic hydrolysis is fragmentation of aglucone into elemental sulfur and nitrile, which depends on the concentration of H^+ or on the presence of other constituents, e.g. Fe^{2+} (Kaoulla *et al.*, 1980).

Isothiocyanates are associated with the characteristic odor and taste of species within the genus Brassica. There is, however, great variation in terms of stability, volatility and pungency of these compounds (Appelquist, 1972).

Isothiocyanates that are hydroxy-substituted in the 2- or 3-position of their side chains spontaneously cyclize into stable nonvolatile oxazolidinethiones (see Figure 2.2).

According to Olsen *et al.* (1980), glucosinolates have near planarity around the double bond, i.e. the oxygen atom, nitrogen atom, C-0, thioglucosidic sulfur atom and C-1, are almost coplanar. The sulfate group strongly imparts acidic properties to the glucosinolates. The very low pKa value of this group (1.99) implies that glucosinolates occur in nature as anions and, because of the instability of glucosinolates in strongly acidic solutions, it is necessary to handle these compounds as salts.

The sulfate group and the thioglucose moiety impart nonvolatile and hydrophilic properties to all glucosinolates. The R-group, although perhaps always derived from amino acids, varies in its properties from lipophilic to markedly hydrophilic. This is of importance in choosing a proper method for analysis of glucosinolate degradation products (Olsen, 1980).

Isolation of intact glucosinolates requires that some metals and strongly acidic and basic conditions be avoided. Olsen *et al.* (1980) described a technique in which a strongly acidic ion-exchange column was connected in series to a weakly basic Ecteola anion-exchange column. The latter has tertiary amino groups (pKa ca. 7.5) as functional groups, which permits the elution of the total pool of glucosinolates by removal of the positive charge of the Ecteola column when 1 M pyridine is used as eluant. This eluant is volatile and the glucosinolates are isolated as pyridinium salts.

Intact glucosinolates have a UV absorption maximum at 235 nm due to the glucosinolate group. This absorption can be used for their detection by HPLC (Olsen *et al.*, 1980). Furthermore, some glucosinolates may have UV absorption at other wavelengths due to the other R-groups. Trimethylsilylation of glucosinolates isolated as pyridinium salts by ion-exchange chromatography results in volatile derivatives of desulfoglucosinolates which can be easily separated and quantitated by GLC. According to Olsen *et al.* (1980), this method allows quantitative analysis of even small amounts of glucosinolates in leaves or other plant parts.

2.2.3.2 Biosynthesis

From the experimental work of Ettlinger and Kjaer (1968) and Larsen (1973), it was established that α-amino acids (Figure 2.3) are subjected to several oxidation reactions, accompanied by decarboxylation and followed by

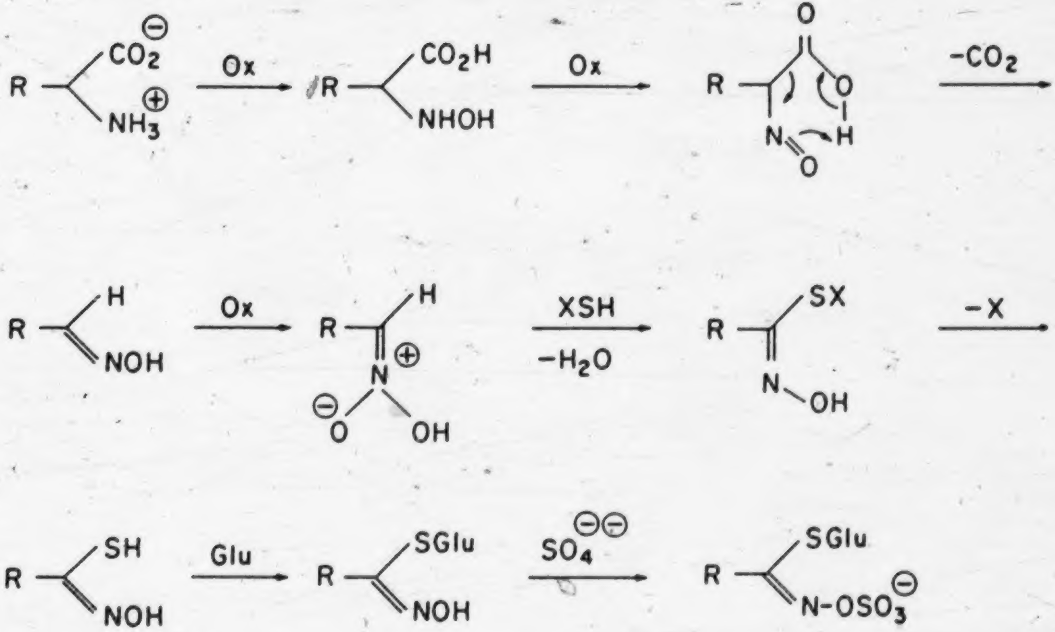

Ox
Ox
$-CO_2$
Ox
XSH
$-H_2O$
$-X$
Glu
SO_4^{2-}

Figure 2.3 Suggested pathway of glucosinolate biosynthesis.

incorporation of sulfur, glucose and, finally, sulfate to yield glucosinolate.

The structures of the side chains of glucosinolates from natural sources suggests that some of these chains are similar to the common protein short chain amino acids, whereas others, the majority, resemble the naturally-occurring α-amino acids with elongated chains.

Glucosinolates derived from common protein amino acids

Aliphatic side chains

Glycine-derived glucosinolate (R=H, Figure 2.3) has not been found, possibly due to its instability. Methylglucosinolate (R=CH3), the alanine derivative, is absent in Cruciferae as well (Kjaer, 1970). Isopropylglucosinolate and butylglucosinolate, originating from valine and L-isoleucine respectively, are frequently found in Cruciferae. The leucine counterpart, isobutylglucosinolate, has been found in some genera (Underhill and Kirkland, 1972). Glucosinolates corresponding to glycine, serine, threonine, cysteine, methionine, lysine, arginine, aspartic acid and glutamic acid have not yet been found in natural form.

Aromatic and heterocyclic side chains

Benzyl- and p-hydroxybenzylglucosinolate, which are derived from tyrosine and phenylalanine, have been identified in Cruciferae (Ettlinger and Kjaer, 1968).

The tryptophan counterpart, 3-indolylmethylglucosinolate, is limited in its occurrence to seedlings and young vegetative tissue of numerous families, including Cruciferae, within which the Brassica genus is known as a useful source (Eliott and Stowe, 1971).

Glucosinolates derived from α-amino acids

A characteristic feature of glucosinolate side chains is the abundance of moieties representing higher homologs [R-(CH_2)-] of those arising directly from protein amino acids, as discussed above. The initial step in biosynthesis of glucosinolates can therefore be, amino acid elongation, as shown in Figure 2.4 (Kjaer, 1973), followed by a reaction sequence (Figure 2.3).

In addition to linear elongation, oxidation of side chains arising from several protein amino acids (e.g. valine, leucine, isoleucine, methionine, phenylalanine and tyrosine) must also be considered in accounting for the complete list of glucosinolates (Kjaer, 1973).

2.2.3.3 Nutritional value of rapeseed meal with respect to its glucosinolate content

The major concern in using rapeseed meal as a partial or sole source of animal feed has been the high content of enzymatically- or chemically-hydrolyzed glucosinolates.

Glucosinolates remain unchanged in intact seed, but upon crushing and cell disintegration, the myrosinase present in the seed splits the glycosidic linkage, giving rise to aglucone, sugar and sulfate (Josefsson, 1972). The molecular rearrangement of aglucone is dependent on

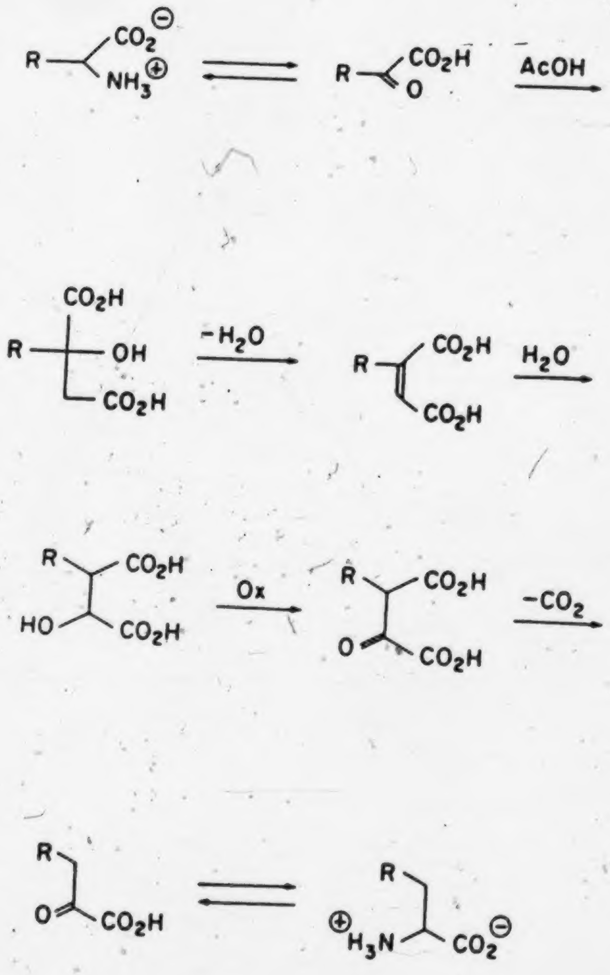

AcOH
-H₂O
H₂O
Ox
-CO₂

Figure 2.4 Amino acid chain elongation.

conditions of storage and treatment of the seed (Van Etten, 1966). This author found that, if the seed had not been stored or heat treated, or if the hydrolysis took place at pH 5 with relatively small amounts of moisture, nitrile formation predominated. The formation of oxazolidinethiones was favored when the seed was stored for a longer time, heat treated (100-120°C) or when hydrolysis was carried at increased temperature, with higher moisture content and at pH>5.

Antinutritional properties of rapeseed meal have been extensively studied. Kennedy and Purves (1941) reported enlarged thyroids in rats fed rapeseed. The conclusion drawn from this and other studies (Matsumoto, 1969; Nordfelt, 1954) was that feeding rapeseed causes thyroid enlargement by interferring with the synthesis of thyroxine in such a way that the anterior pituitary is stimulated to produce thyroid-stimulating hormone (TSH), which causes hypertrophy and hyperplasia of the thyroid gland.

Astwood (1949) isolated a goitrogenic substance from rapeseed which was shown to be 5-vinyloxazolidinethione (5-VOT).

Poor palatability of rapeseed meal has been associated with volatile isothiocyanates, namely 3-butenyl, 4-pentenyl and phenylethyl-isothiocyanate (Josefsson, 1972). Van Etten *et al.* (1969) found that rats fed meals with a higher content of nitriles showed thyroid enlargement and microscopic lesions in the liver and kidneys.

Different approaches in counteracting toxicity of rapeseed meals (Appelquist and Josefsson, 1972; Bell and Belzile, 1965; Sosulski *et al.*, 1972; van Megen, 1983) gave results of limited success. The ultimate choice was in breeding for rapeseed cultivars low in glucosinolates (Stefansson and Kondra, 1975). New cultivars with only 10% of the glucosinolate content of "normal" rapeseed significantly improved palatability and nutritional value of rapeseed meal. Clandinin *et al.* (1983) concluded that up to 15% canola meal can be used as partial replacement of soybean meal in isocaloric, isonitrogenous rations for laying chickens without adverse effects on egg quality, mortality of animals and thyroid gland function. Hawrysh *et al.* (1983) reported that inclusion of 20% canola meal into the chicken diet did not have any effect on the palatability of cooked chicken meat.

Campbell and Cansfield (1983) indicated that feeding roosters with rapeseed meal containing intact glucosinolates (IG) resulted in excretion of only 1/3 of the latter. Apparent breakdown of ingested IG was only partly attributed to microorganisms in the intestinal tract.

Cansfield and Campbell (1983) reported that reduced capability of liver for detoxification of foreign compounds can be related to the formation of coordination complexes of 5-VOT with metals, which in turn inhibit the oxidation processes of liver mixed-function oxidase enzymes.

Bell *et al.* (1980) proposed that a slight decrease in performance of pigs fed 15% canola meal was due to reduced energy digestibility, but not to glucosinolates. Similar results were obtained from experiments on dairy cattle (Sharma *et al.*, 1980; Chase *et al.*, 1980).

Although goitrogenicity of canola meal is very much less for meals produced from older rapeseed cultivars, increased thyroid weight has still been observed (McGregor, 1978). According to the author, this can be related to thiocyanate ion (SCN^-), which is known to be a breakdown product of the indole glucosinolates, glucobrassicin and neoglucobrassicin. These relatively unstable compounds cannot be detected by gas chromatographic or spectrophotometric analytical methods. Thus, while the overall reduction of glucosinolates in double zero cultivars may be over 90%, the level of thiocyanate ion precursor remains effectively unchanged.

McGregor (1980) reported that gluco- and neoglucobrassicin comprise 6-13% of the total glucosinolate content in high glucosinolate rapeseed and over 40% in certain canola meals. Fenwick (1980) concluded that canola meals, with their improved palatability and nutritional effects, can be fed at levels approaching double that of high glucosinolate rapeseed meal without adverse effects.

The finding of McGregor (1978) necessitated the development of analytical technique(s) in which glucosinolates are monitored in their intact form. This was accomplished by Helboe *et al.* (1980), using a reverse phase, ion pair HPLC system with a Nucleosil 5 C18 column and 0.01 M phosphate buffer (pH 7):methanol (3:7, v/v), containing 0.05 M tetraheptylammonium bromide, as a mobile phase. Glucosinolates were isolated on an ion-exchange column by the method of Olsen and Sorensen (1979) as pyridinium salts and injected on the HPLC column as 0.01-0.04% aqueous solutions. Different k' values for IG when using an appropriate counter ion provided satisfactory separations.

Mullin (1978) reported that methanol was not an appropriate solvent in gas chromatographic analysis of organic isothiocyanates due to formation of addition compounds with methanol. This was confirmed by mass spectral analysis in which compounds with molecular ion species corresponding to each standard isothiocyanate molecular weight + 32 were detected.

2.2.4 Other seed constituents

2.2.4.1 Enzymes

According to Appelquist (1972), lipase, lipoxygenase and thioglucoside glucohydrolase (myrosinase) are the most important enzymes in rapeseed. Other enzymes found in dormant seed are choline kinase, glyceric acid kinase and glutamic acid decarboxylase. Lipase activity is low in dormant seed but increases rapidly at the onset of germination and is 100 times that of dormant seeds (Wetter, 1957). During the germination, lipase activity of *B.*

campestris extracts was 5 times greater after 5 and 7 days than *B. napus* extracts, a fact which may be of technological significance.

Tookey and Wolff (1964) studied the specificity of lipase in *Crambe abyssinica*. It was shown that the enzymic attack was essentially random, with some preference for palmitic acid and nonpreference for erucic acid.

Little work has been done on rapeseed lipoxygenase. Appelquist (1972) suggested that the lack of lipoxygenase activity in dormant seed could be due to the presence of inhibitors. St. Angelo and Ory (1984) investigated the activity of lipoxygenase in four rapeseed cultivars differing in the content of erucic acid (0.2-54%). They concluded that even minute concentrations of erucic acid (0.2%) had an inhibitory effect on lipoxygenase activity.

Myrosinase (thioglucoside glucohydrolase)

"Myrosinase" is the collective name for several isoenzymes present in rapeseed. Bjorkman and Lonnerdal (1973) achieved the separation of four isoenzymes, designated RA, RB, RC and RD, by the procedure described by Lonnerdal and Janson (1973). Furthermore, the major component, RC, could be separated into three homogenous components, designated as RC1, RC2, and RC3. The authors assayed the activity of these isoenzymes in terms of pH optima, temperature maxima and stability at various pH's and temperatures. The pH optimum interval was found to be between pH 4 and 5. A temperature of 60°C was reported to be optimal for all four isoenzymes. Maximal stability for the enzymes was at pH 6 and 4°C. After six months of storage, the enzyme activity was unchanged. When the purified isoenzymes and the crude rapeseed extract were exposed to different temperatures at pH 6, it was found that the activity fell rapidly at 37°C as well as at 25°C during the first two weeks. It was noticed however, that the purified enzymes were more stable than the crude preparations. Freezing, contrary to expectations, completely destroyed the activity of the purified enzymes, but did not significantly affect the activity of the crude extract. After the observation that imidazole buffer at pH 6 was preferred to citrate buffer at pH 5.5, the authors recommended that the best way of storing myrosinase was in imidazole buffer at pH 6 and 4°C.

In analyses of the effects of ascorbic acid on myrosinase activity, it was found that the rate of sinigrin (used as a substrate) hydrolysis increased (Bjorkman and Lonnerdal, 1973). The maximum activity was found at an ascorbic acid concentration of $3x10^{-3}$ M. At higher concentrations, ascorbic acid functioned as an inhibitor.

Experiments on interspecies differences in myrosinase activity revealed that the average activity in *B. napus* was higher than that in *B. campestris*, but the activation with ascorbic acid behaved in the opposite manner, (Bjorkman and Lonnerdal, 1973).

Ohtsuru and Hata (1978) found that L-ascorbic acid activated plant myrosinase, whereas ascorbic acid analogs (such as D-araboascorbate, glucoascorbate, etc.) did not. L-ascorbic acid also affected the optimum temperature for the enzyme activity so that in its presence the optimum temperature was 35°C, while in the absence of L-ascorbic acid, the optimum temperature was 55°C. In addition, it was found that the activation mechanism of myrosinase by L-ascorbic acid depended on a conformational change in the enzyme protein moiety induced by L-ascorbic acid, and not on the dissociation and association mechanism of myrosinase. In the mechanism of action of the enzyme, the authors postulated the presence of one active site for the substrate and two sites for ascorbic acid. The substrate site has two loci, one for the glycon and one for the aglycon part of glucosinolate. The conformation of the binding loci for the aglycon moiety is altered when the ascorbic acid site is occupied. Since myrosinase activity was strongly inhibited by Na-p-chloromercuribenzoate (specific inhibitor of sulfhydryl groups), with or without ascorbic acid, sulfhydryl groups were found to be essential for the catalytic action of myrosinase.

Inactivation of myrosinase with an amino group inhibitor (2-methoxy-5-nitrotropone) was effective without addition of ascorbic acid, but in its presence enzyme activity was not affected. Based on these facts, it was concluded that the amino residues were situated in the region altered by the addition of L-ascorbic acid (close to aglycon moiety) and their locations were changed to accelerate the enzyme action by the binding of ascorbic acid to the effector site.

Kozlowska *et al.* (1983) emphasized the unsuitability of Schwimmer's (1961) method for isolation of rapeseed myrosinase, because only about 50% of the enzyme could have been extracted with 30% acetone. Complete extraction was achieved with 0.9% NaCl in 0.05 M phosphate buffer pH 7.0.

The same authors obtained two fractions of rapeseed myrosinase by precipitation with ammonium sulfate (40% and 70% saturation) of the supernatant of dialysed and centrifuged solution of crude extract in the salt phosphate buffer, pH 7. These two fractions exerted different responses to the addition of ascorbic acid. In experiments with rapeseed meal, fraction II (obtained after 40-70% saturation with ammonium sulfate) responded much more to the presence of ascorbic acid than did fraction I (after 40% saturation).

In the above study, separation of two molecular forms of enzyme (MI and MII) was achieved after seven purification steps of crude extract, on a QAE A-50 Sephadex gel column (H = 30 cm, i.d.= 2.5 cm) with linear gradient of 0.05-0.3 M K-phosphate buffer of pH 6.7. Forms MI and MII differed in isoelectric points (6.2 and 5.7) and K_m using sinigrin as substrate hydrolysis (0.093 mmole and 0.053

mmole). Also, they differed in maximal activation with ascorbic acid (0.28 mmol and 0.56 mmol) and optimal pH stability (4.0-7.5 and 5.5-8.0), while the optimum temperature was 55°C for both molecular forms.

In assessment of the activity of the crude enzyme extract at elevated temperatures combined with high relative humidity, Kozlowska *et al*. (1983) found that, in the intact and flaked seeds, myrosinase inactivation began after 5 min of incubation at 90-100°C and at a relative humidity of 90-100%. At lower temperature and lower humidity, the enzyme was more easily inactivated in the flaked than in the intact seeds. However, in the whole seed, there was no evidence of quantitative changes of glucosinolate levels. On the contrary, in the flaked seed some hydrolysis of glucosinolates occurred. This was explained by the fact that in flaked seeds myrosinase was already in contact with its substrate, glucosinolates, and before its inactivation it hydrolyzed a substantial amount of glucosinolates.

2.2.4.2 Pigments

The natural color of rapeseed oil originates from the presence of lipochromes, i.e. carotenoids and pigments such as chlorophylls and their degradation products, pheophytins (Persmark, 1972). Structural properties of these compounds make their presence easily measurable by various spectrophotometric methods. Box *et al*. (1967) isolated carotenoids from rapeseed by solvent extraction after saponification and subsequent partitioning between petroleum ether and 90% methanol followed by on-column and thin-layer chromatography. The authors reported neo-lutein A and B, 23 ppm, as the major carotenoids, while carotene was 1.8 ppm. Pheophytin A was the major component of the chlorophylls at 7.6 ppm, while chlorophylls A and B only amounted to 0.3 and 0.4 ppm, respectively.

Niewiadomski *et al.* (1965) analyzed the content of chlorophylls A and B and pheophytins A and B in rapeseed oils at four different wavelengths, i.e. 663.8, 645, 668 and 655.8 nm, respectively. The main component found was pheophytin A, at 18-26 ppm. The carotenoids, isolated on alumina columns, were measured at 450 nm. β-carotene was used as a reference since it was found to be the major component of the oil. Benk *et al.* (1965) reported 2.1-2.9 ppm carotenoid content in rapeseed oil, 70-75% of which was β-carotene.

2.2.4.3 Proteins and amino acids

Rapeseed proteins, according to Appelquist (1972), are characterized by a structural function (membrane components), a catalytic function (membrane-bound or free), and/ or a storage function. In rapeseed, the storage protein is located in specific organelles, so-called protein bodies or aleurone grains (von Hofsten, 1970).

Amounts of free amino acids and peptides are very low in mature rapeseed (Finlayson, 1967). Some enzymes are membrane bound, but the majority are free within the cytoplasm.

The contents in canola meal of protein, and its amino acid composition are comparable to those of soybean meal (Clandinin, 1981). Canola meal is somewhat lower in lysine but substantially higher in methionine content than soybean meal (Table 2.6).

Numerous researchers have analyzed the rapeseed protein obtained by extraction with different solvents. Kodagoda *et al.* (1973) applied a three stage extraction consisting of water, dilute acid and dilute alkali and obtained extracts with 11, 13 and 42% protein yield, respectively. The authors reported higher levels of lysine, histidine and arginine from aqueous and acid extracts. Finlayson *et al.* (1976) reported the extraction rate with dilute alkali (0.1 M NaOH) to be 85-90% of the meal nitrogen. This study also dealt with the effect of different solvents used for extraction on the structure of 12 S globulin, which is the major protein of aleurone grains, and on the 1.7 S protein fraction. Relative stability of 12 S globulin was observed in the pH range 8.0-12.0, and the intermolecular disulfide bonds were found not to have a major effect on the recovery of the globulin from the meal.

The high content of 12 S globulin in the rapeseed explains, according to Goding *et al.* (1970), increased extractable nitrogen (N) above pH 8, since the former protein species is soluble in alkaline, but not in neutral aqueous solutions. SDS electrophoresis, however, showed no evidence of the presence of high molecular weight proteins.

Table 2.6 Protein contents and amino acid compositions of canola and soybean meals[a].

	Canola meal		Soybean meal	
	As fed (%)	In protein (%)	As fed (%)	In protein (%)
Proximate composition				
Moisture	7.49		11.00	
Crude fiber	11.09		7.3	
Ether extract	3.78		0.8	
Protein (Nx6.25)	37.96		45.01	
Amino acid composition				
Alanine	1.73	4.56	1.89	4.20
Arginine	2.32	6.11	2.90	6.44
Aspartic acid	3.05	8.03	5.04	11.20
Cystine	0.47	1.23	0.29	0.65
Glutamic acid	6.34	16.69	8.10	18.00
Glycine	1.88	4.96	2.07	4.60
Histidine	1.07	2.81	1.08	2.40
Isoleucine	1.51	3.98	2.11	4.69
Leucine	2.65	6.97	3.37	7.49
Lysine	2.27	5.98	2.80	6.22
Methionine	0.68	1.78	0.63	1.40
Phenylalanine	1.52	4.01	2.16	4.80
Proline	2.66	7.00	2.20	4.89
Serine	1.67	4.39	2.25	5.00
Threonine	1.71	4.50	1.71	3.80
Tryptophan	0.44	1.16	0.54	1.20
Tyrosine	0.93	2.46	1.26	2.80
Valine	1.94	5.11	2.25	5.00

[a] From Clandinin (1981).

Incubation conditions of 4 M urea and 1% SDS dissociated the 12 S protein into subfractions which were not noticeably different in molecular weight from the proteins extracted by water alone at neutral pH. The same conclusion was reached by Quinn *et al.* (1976), who resolved over 30 different proteins from *B. campestris* cv. Echo by gel electrofocusing. They also studied the impact of different solvents (5% NaCl and 5% $CaCl_2$) on N-extractability over the pH range of 2.5 to 11.0. While both ionic environments increased the solubility of the protein over the wide pH range, neither method suggested practical possibilities for protein enrichment.

Kodagoda *et al.* (1973) analyzed functional properties of protein isolates and concentrates, obtained by successive water, HCl and NaOH extractions in baking, emulsification and whipping tests. Addition of protein isolate from aqueous and HCl extraction at a level of 5% led to 10-14% larger loaf volume, whereas addition of concentrates gave poorer results. Whipping tests with 3% replacement of egg white protein by isolates or concentrates resulted in decreased specific volume compared to all-egg white control, with the exception of HCl-extracted isolate, which had a 10% larger specific volume than the control. The highest emulsification capacity was observed with water extracts. Thompson *et al.* (1982) confirmed these findings, but reported poor water absorption and gelling properties for canola protein concentrate.

According to Goh *et al.* (1980), canola protein solubility in dilute alkali should not be used as a criterion for the protein quality. Results of feeding trials with chicken did not correlate well with the data from the former chemical test.

2.3 Effects of Processing on Canola Oil and Meal Quality

2.3.1 Flaking

The general outline of canola oil and meal production is given on Figure 2.5. The aim of flaking, according to Beach (1983), is to obtain an optimum surface/volume ratio of crushed seeds in order to maximize the oil yield from subsequent pressing and/or solvent extraction. Work of Othmer and Agarwal (1955) on countercurrent batch extraction elucidated the relationship between the extraction rate on one side and the flake thickness and residual oil on the other. The extraction rate was found to be inversely proportional to the flake thickness (-3.97 power) and directly proportional to the concentration of the residual oil (3.5 power). In the case of canola, the best results were obtained with flakes of 0.2-0.3 mm size. Production of flakes of uniform thickness is greatly affected by the preadjustment of seed temperature and moisture content (Beach, 1983).

An additional reason for moisture adjustment (6-9%) is that, upon crushing, myrosinase would promote glucosinolate

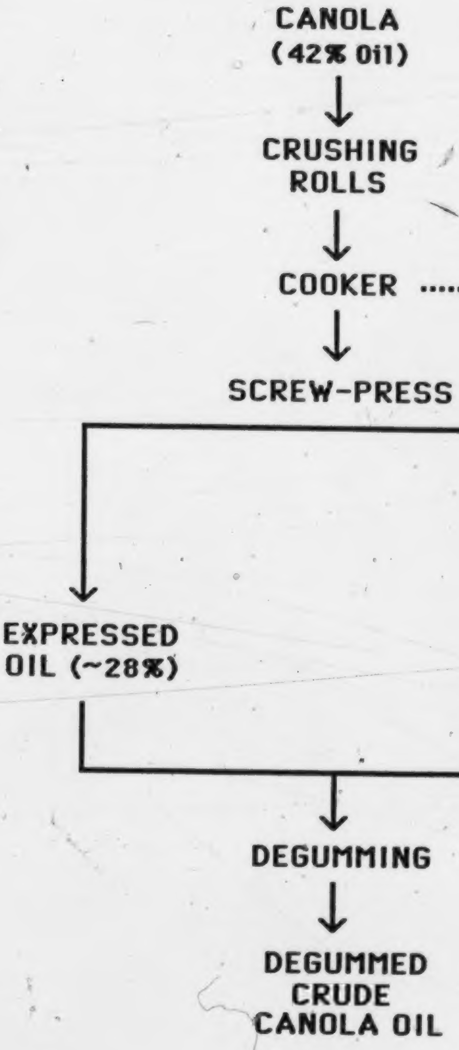

CANOLA
(42% Oil)
CRUSHING ROLLS
COOKER
SCREW-PRESS
SOLVENT EXTRACTOR
EXPRESSED OIL (~28%)
EXTRACTED OIL (~14%)
DEGUMMING
DEGUMMED CRUDE CANOLA OIL

Figure 2.5 Outline of the primary processing of canola.

hydrolysis if the moisture of the seed was too high (Anjou, 1972; Weinberg, 1972).

2.3.2 Cooking

Heat-treated oilseeds release their oil more readily than uncooked seeds, although the exact reason and complete explanation of this fact are obscure (Norris, 1979; Beach, 1983). Bernardini (1982) explained this phenomenon as follows:

- increase in temperature causes coalescence of ultramicroscopic oil droplets and their flow out from the cells is thus facilitated.
- heat denaturation of proteins breaks down the oil-protein emulsion and the recovery of the oil is more readily achieved in pressing and extraction.

Anjou (1972) added insolubilization of phosphatides, completion of the breakdown of oil cells, reduction of the affinity of the oil for the solid surfaces, inactivation of enzymes, drying the seeds to a suitable moisture content for efficient solvent extraction, and destruction of molds and bacteria as major objectives of rapeseed cooking.

The process is usually carried in stack cookers (Beach, 1983) which consist of a series of closed cylindrical steel kettles (5-8) stacked one on top of the other. Each kettle is equipped with a sweep-type stirrer for agitating the crushed seeds during cooking. The kettles are jacketed for indirect steam heating. Automatic gates provide a continuous gravity flow of the seed down through the kettles.

Anjou (1972) classified major operation parameters of rapeseed cooking into residence time, temperature and moisture. Dry heating was an obvious choice for rapeseed cultivars high in glucosinolates in order to prevent their enzymatic hydrolysis.

Weinberg (1972) reported that, depending on plant design, steam pressure, seed temperature etc., temperatures as low as 30°C have been observed in the first kettle (tray), although it is feasible for seed to reach at least 55-60°C.

As suggested by Beach (1983), rapid heating of rapeseed flakes through 50-80°C would minimize glucosinolate hydrolysis by myrosinase. The activity of this enzyme, according to Bjorkman and Lonnerdal (1973), rises rapidly at temperatures >30°C, reaching maximum activity at 50-60°C, while inactivation of the enzyme occurs at 80-85°C. However, these data do not apply if the seed moisture is <6%, and, even though the myrosinase activity is minimal under this condition, the enzyme resists heat inactivation at temperatures of 88-93°C (Beach, 1983). Another disadvantage of low seed moisture is that the seed shatters during flaking. The optimum temperature for lipase is 37-40°C and inactivation with heat depends mostly on the moisture levels in the seeds (Anjou, 1972). Commercial processing largely depends on the moisture levels in the seeds (Anjou, 1972). Commercial processing therefore employs rapid temperature rise of the flakes containing adequate moisture content (6-8%) beyond deactivation temperature(s) (Beach, 1983).

Eapen (1968) performed various laboratory heat treatments in order to evaluate their effect on myrosinase stability. Dry heat treatment (30 min, 105°C) proved unsatisfactory, but steam blanching (5 min), microwave heating (3 min) or immersion of the seed in boiling water (1.5 min) were effective.

If the moisture content of the seed material is high and the temperature rise is not rapid enough, glucosinolates may be hydrolysed before the myrosinase is inactivated (Reynolds *et al.*, 1964). Heat treatment may also have negative effects on oil and meal quality. If cooking temperatures above 110°C are applied, the extracted oil may be difficult to hydrogenate. This has been attributed to chemical breakdown of glucosinolates to give oil-soluble sulfur-containing compounds (de Man *et al.*, 1983; Reynolds *et al.*, 1964).

The aforementioned experiments by Eapen (1968) with microwave heating and steam blanching gave dark colored oils, while dry heat treatment and immersing into the boiling water gave lighter colored oils. A lowering of oxidation stability may take place during heat treatment of the seeds, leading to an increased peroxide value of the oil (Appelquist, 1967).

According to Norris (1979), the content and the degree of activation in oilseeds of surface-active substances, namely free fatty acids and phosphatides, influences the tendency of the seed to absorb and retain the oil. This is supported by the fact that damaged seeds give lower oil yields, probably due to their higher content of free fatty acids or some other surface-active agents.

Bernardini (1983) emphasized the relationship between temperature and moisture content during cooking, stressing that the optimum conditions for moisture and temperature must be found for each seed species in order to obtain maximum yield of acceptable grade oil during mechanical and/or solvent extraction. Norris (1979) theorized that heating of the seed with sufficient moisture content produces a film of water on the seed surface, which favors the diffusion of the oil out of the seed. The oil-bearing cells are made permeable to the oil only by the action of heat and moisture and yield the oil upon the treatment with solvents, which would not be obtained even after the most careful reduction of the seed size because many of the cells still remain intact. In addition, the author proposed that the water may be in a more nearly bound state, and its presence in this condition may serve to make the seed surface relatively lipophobic. Beach (1983) found that, on completion of cooking of canola flakes, the moisture levels of 2.75-3.5% provided for satisfactory pressing and contributed to a press cake of good quality.

2.3.3 Screw pressing

Extraction of rapeseed oil by means of screw presses can be done as straight pressing or prepressing for subsequent solvent extraction. Youngs (1965) reported that, in the former case, oil content of the cake was 4-7% and in the latter 12-20%.

The process of straight pressing, as stated by Anjou (1972), has the advantages over solvent extraction of being simple and requiring lower investment cost. Disadvantages are, however, high power consumption and wear and tear on the machinery. Another major drawback, as reported by Dunning (1956), is that it is only possible to press to a residual oil content of 4-7%, resulting in high losses in yield. Poor oil and meal quality from the straight pressing method lead most plants to adopt the prepress-solvent extraction method for oil recovery (Anjou, 1972). After pressing, the oil contains fine meal particles. Before further treatment, the oil has to be settled in a tank and filtered in filter presses with added filter aids (Youngs, 1965). The amount of fines, and thus the filtration rate, is highly dependent upon the cooking process (Anjou, 1972).

2.3.4 Solvent extraction

Extraction studies by Karnofsky (1949) brought up the conclusion that the bulk of the oil is extracted rapidly, in contrast to the slow extraction of the remainder of the oil. Two different mechanisms have been proposed to explain the extraction phenomenon (Becker, 1964). These are the theory of molecular diffusion and the theory of functional dependence of extraction rate on the rate of solubilization of undissolved oil.

Counter-current extraction is accepted today as the most efficient way of oil extraction. The most widely used solvent is n-hexane, a petroleum fraction with b.p. 63-66°C, refined for use in the vegetable oil extraction industry.

Although elevated temperatures reduce the oil viscosity and enhance diffusion, hexane vapor pressure limits the practical operating temperature of extraction to about 55-60°C. Higher temperature and, consequently, higher vapor pressure increases the volume of the vapor, thus imposing higher recovery and solvent costs. An advantage of double zero cultivars is the possibility of applying lower cooking temperatures, which, in addition to the reduced energy consumption for cooking, eliminates the necessity of cake cooling prior to solvent extraction, (Beach, 1983).

Aqueous extraction of rapeseed oil on a laboratory scale was attempted by Embong *et al.* (1977). Compared to Soxhlet-extracted oil, aqueous extracts were lower in free fatty acid and phospholipid content, but had higher sulfur concentration and increased peroxide value. The overall disadvantage of the method was in its poor oil yield, which, at maximum, was just slightly over 90%.

2.3.5 Oil degumming

The crude solvent-extracted oil contains about 2% polar lipids, often referred to as "gums" or "lecithin", which have to be removed in the process of degumming if difficulties in handling of the oil and high refining losses are to be avoided.

The oil is treated with a small amount (1-4%) of water or with dilute aqueous solutions of phosphoric or citric acid, which allows insolubilization of hydratable phospholipids and their subsequent removal via centrifugation (Norris, 1979). Nonhydratable phosphatides or β-lipoids (Carr, 1978), however, cannot be removed by the water-degumming process. Since rapeseed phospholipids have been found to be inferior emulsifiers in terms of color and taste, as compared to soybean lecithin, they are mixed, after centrifugation, with the de-oiled meal in which they act as a dust suppressant and as a binder in meal pelleting (Beach, 1983).

In some instances in the degumming process, coagulants are added to the reaction mixture (Leibowitz *et al.*, 1980) to facilitate centrifugal separation of the phosphatide phase. Dahlen *et al.* (1980) approached the problem of achieving better degumming by using a higher seed conditioning temperature of 100°C, instead of 85°C, in the stack cooker to inactivate phospholipases. These enzymes are responsible for the formation of the nonhydratable phosphatide breakdown products (Ong, 1981).

The oil extracted from seed conditioned at the higher temperature is higher in phosphatides, but water degumming removes these to the very low levels achieved otherwise only in acid degumming. Disadvantages are that the concentration of sulfur compounds in the oil is raised and the residual oil content of the meal tends to be higher.

2.3.6 Meal desolventizing

In the desolventizing process the hexane is removed from the meal. In addition, protein denaturation and reduction in volatile glucosinolates breakdown products takes place. Most of the heat required in the desolventizing process is supplied by the condensing steam, which saturates the meal with moisture in place of the displaced hexane. Steam, in addition to that which condenses, provides a partial pressure effect on the evaporating hexane, lowering its effective boiling point and enhancing its removal from the meal (Beach, 1979). A supplemental toasting process produces well-flavored dark-colored meals due to nonenzymatic browning reactions (Norris, 1979).

Anjou (1972) described a flash desolventizing system which minimizes protein losses and produces a meal of lighter color by employing mild heating conditions with minimum retention time.

3. MATERIALS AND METHODS

3.1 Seeds

Canola seeds used in all experiments were a mixture (60:40, w/w) of two Alberta grown cultivars, Tobin (*B. campestris*, L.) and Westar (*B. napus*, L.). Seeds were supplied by Alberta Food Products (AFP), Fort Saskatchewan. The use of the mixture of two cultivars, one being high in oil and protein and low in fiber contents (Tobin) and the second being high in fiber content (Westar) is preferred by AFP for the optimum balance between high oil and protein yield and handling characteristics of the seeds during processing.

3.2 Chemicals

Silicic acid, 100 mesh, chromatography grade, was from Mallinckrodt (Montreal, Qué.). Silica gel, type G, 10-40 µm particle size, and lipid standards were supplied by Sigma Chemical Co. (St. Louis, MO). Glutaraldehyde (10%), formaldehyde and osmium tetroxide were from Stevens Metallurgical (New York, NY). NMA (nadic methyl anhydride), DDSA (dodecenyl succinic anhydride) and DMP-30 (dimethyl aminomethyl phenol), uranyl acetate and lead citrate were from Ladd Research Industries, Inc. (Burlingon, VT). All other chemicals were of reagent grade and were supplied by Fisher Scientific Co. (Fair Lawn, NJ) or by BDH Chemicals (Toronto, Ont.).

3.3 Equipment

Goldfish apparatus (Labconco Corp., Kansas City, MO) for oil extraction with n-hexane.

Oxygen bomb from Parr Instruments Co. (Moline, IL).

Spectrophotometers: Varian model DMS 90 with Varian Cary batch sampler (Varian, Walnut Creek, CA); Beckman DU-8 UV-visible computing/scanning spectrophotometer (Beckman Scientific Instr., Irvine, CA); Spectronic 20 (Bausch and Lomb, Inc., Rochester, NY).

Thermolyne Dri-bath from Thermolyne Corp. (Dubuque, Iowa).

Fisher Isotemp forced draft oven from Fisher Scientific (Fair Lawn, NJ).

Vacuum oven from GCA Corp. (Chicago, IL).

Steam bath from Gallenkamp Co. (England).

Vacuum rotary evaporator, model Rotavapor-R, from Büchi Glasapparate Fabrik (Flawil, Switzerland) with Thermolift water bath (Buchler Instr., Fort Lee, NJ).

Cahn Electrobalance from Ventron Instr. Co. (Paramount, CA).

TLC spreading unit and glass plates from Desaga (Heidelberg, W. Germany).

Hoefer transmittance/reflectance scanning densitometer, model GS-300, from Hoefer Scientific Instr. (San Francisco, CA) with Cole-Palmer chart recorder from Linear Instr. Co. (Irvine, CA).

Incubator, model 1000FAATR, from Cleland International Inc. (Rogers, MN).

Laboratory mill, model 4E, from Straub Co. (Philadelphia, PA).

Hydraulic press from Fred S. Carver Inc. (Summit, NJ).

Centrifuges: Beckman J-21B with JA-20 rotor (Beckman Instr., Spinco Div., Palo Alto, CA); Damon IEC, model HN-SII, from Damon/IEC Div. (Needham Hts., MA); Janetzki T5 (Janetzki, Leipzig, E. Germany).

Scanning electron microscope, Cambridge Stereoscan model 250 (Cambridge Instruments, Inc., Cambridge, England).

Transmission electron microscope, model EM 300, from Philips (Eindhoven, Holland).

3.4 Lipase and Lipoxygenase Activity in Canola Seeds Upon Crushing

3.4.1 Moisture content of canola seeds

Moisture content was determined by the method used by Madsen (1975). Crushed seeds (5 g) were transferred to a preweighed aluminum dish and dried in an oven at 105°C for 16-18 hr. After cooling in a desiccator, the dish was reweighed and the moisture content was calculated from the weight loss.

3.4.2 Sample treatment and lipid extraction

Seed samples (5-10 g) were crushed in a mortar and exposed to holding times of for 0, 5, 10, 15 and 30 min at room temperature, then boiling methanol was added to inactivate the enzymes (Figure 3.1).

Extraction of lipids was achieved by refluxing the samples with chloroform:methanol (2:1, v/v) for 5 min, followed by filtration and reextraction with the same solvent. Filtrates were combined and the solvent was evaporated in a vacuum rotary evaporator at 40°C under nitrogen. Samples were purified from nonlipid contaminants using the method of Folch *et al.* (1957), as follows: Crude lipid residue was redissolved in 150 ml of chloroform: methanol (2:1, v/v), and shaken in a separatory funnel with one-fifth its volume of 0.58% aqueous sodium chloride for 2 min. Equilibrium was allowed to establish overnight at 4°C and the upper phase was removed. Remaining impurities at the interface were rinsed several times with small amounts of the upper phase solvent. The lower phase, containing lipids, was evaporated to dryness under vacuum at 40°C under a stream of nitrogen. Purified lipid samples were dissolved in ethanol-free chloroform and stored in the dark at -18°C.

3.4.3 Lipid fractionation on SiO_2 column

Glass columns 250 mm x 8 mm i.d., with sealed, coarse fritted disc support were used. Silicic acid (3 g; 100 mesh) activated at 120°C for 2 hr and 1 g of Celite were slurried

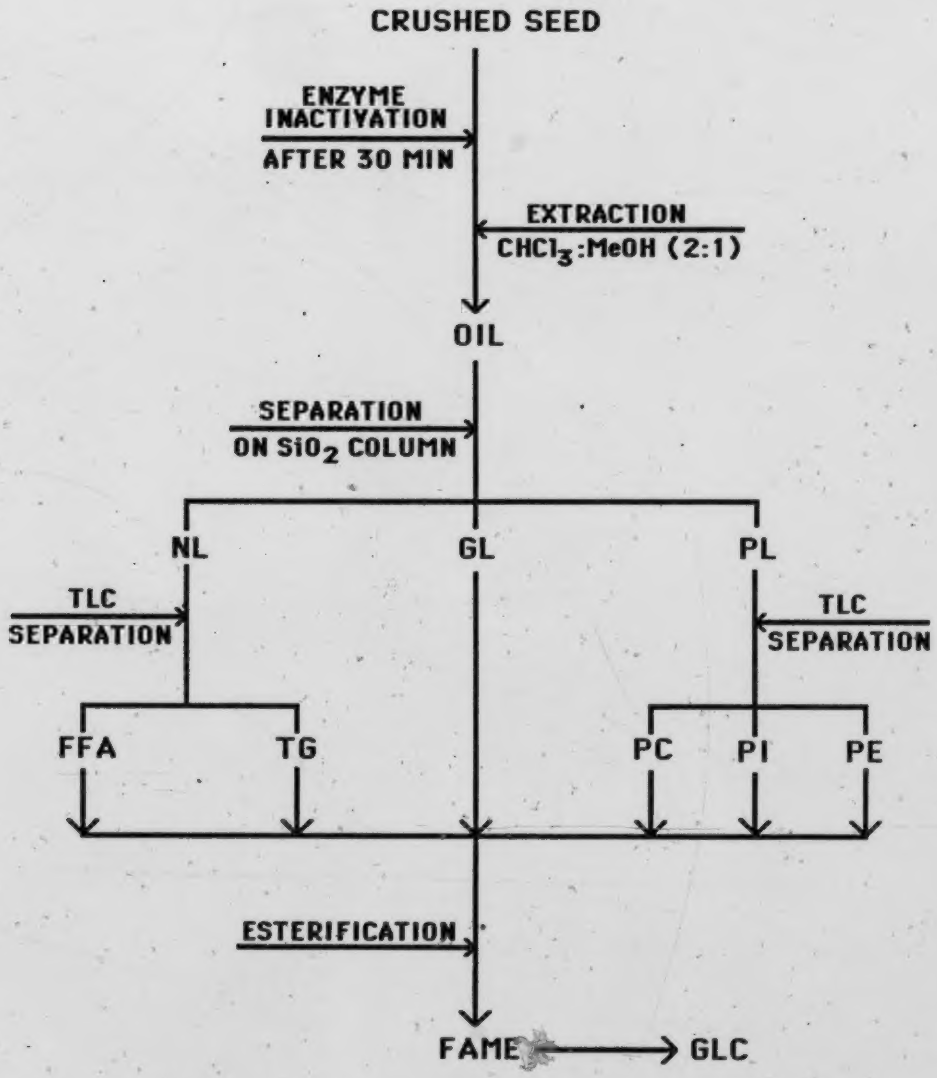

CRUSHED SEED
ENZYME INACTIVATION
AFTER 30 MIN
EXTRACTION
CHCl3:MeOH (2:1)
OIL
SEPARATION
ON SiO2 COLUMN
NL
GL
PL
TLC
SEPARATION
TLC
SEPARATION
FFA
TG
PC
PI
PE
ESTERIFICATION
FAME
GLC

Figure 3.1 Sample preparation for the analysis of lipase and lipoxygenase activity in the seed after crushing.

in diethyl ether and packed into the column. Slurry was added in small portions, allowing the adsorbent to settle uniformly after each addition, until forming a bed with a height:diameter ratio of 10-12:1.

Aliquots of 150-200 mg total lipids were dried under nitrogen and redissolved in 1-1.5 ml diethyl ether. Each sample was carefully applied on the column with Pasteur pipette so that the adsorbent surface was not disturbed. The stopcock was opened to allow the sample to drain into the adsorbent bed, and the walls of the column just above the bed surface were rinsed with small amounts of pure solvent.

Elution of lipid fractions was carried out with solvents of increasing polarity. Neutral lipids were eluted with 80 ml of diethyl ether, glycolipids with 70 ml acetone, and phospholipids with 50 ml chloroform:methanol (1:1, v/v), followed by 40 ml methanol. Each fraction was evaporated to dryness under nitrogen, redissolved in chloroform and determined gravimetrically on a Cahn electrobalance.

3.4.4 TLC separation of lipid fragments

3.4.4.1 Qualitative analysis

For identification of lipid classes, glass plates 20 cm x 20 cm, coated with 0.3 mm Silica Gel G, were used. Lipids (50-200 μg per spot) were applied and the plates were developed in the following solvent systems: neutral lipids in diethyl ether:benzene:ethanol:acetic acid (40:50:2:0.2, v/v) followed by a second development in diethyl ether:

hexane(6:94, v/v); phospholipids in chloroform:methanol: acetic acid:water (170:30:20:5, v/v); and glycolipids in chloroform:acetone:water (30:60:2, v/v). Plates were dried under vacuum at 60°C for 30 min, followed by spraying with 50% sulfuric acid and charring at 170°C for 20 min. Bands were identified by comparing the Rf values with literature data and with standards.

3.4.4.2 Preparative TLC separation of lipid classes for fatty acid analysis

Glass plates coated with 0.5 mm Silica Gel G were used, applying on each plate 15-20 mg neutral lipids and 5-6 mg phospholipids. Glycolipids were esterified directly. Marker lanes on both sides were spotted as well, and the plates were developed in solvent systems as described above. After drying, marker lanes were sprayed with 50% sulfuric acid and heated in an oven at 80°C until the bands became visible. Free fatty acids and triacylglycerols were identified (neutral lipid fraction), as well as phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol (phospholipid fraction). In order to remove the lipids from adsorbent, corresponding bands were scraped with a razor blade and transferred to 150 mm x 8 mm i.d. glass columns with fritted disc support. Neutral lipid fractions were then eluted with diethyl ether, while methanol was used for elution of phospholipid constituents. Samples were evaporated to dryness in a vacuum rotary evaporator at 40°C under nitrogen, and fractions collected were redissolved in

1 ml chloroform and gravimetrically determined on a Cahn electrobalance. Esterifying solution (2 ml; methanol: benzene:acetyl chloride, 20:4:1, v/v) was added to the dried triacylglycerol fraction in 10 ml round bottom flasks and air condensers were attached. Esterification was carried out for 1 hr on a sand bath at 110°C. Fatty acid methyl esters (FAME) were evaporated to dryness under nitrogen and redissolved in 1 ml HPLC-grade n-hexane. FAME analysis was done on a Varian 3700 gas chromatograph equipped with a flame ionization detector. Aliquots of 1.0-3.2 μl were injected and the FAME were separated on a DB Wax, fused silica capillary column, 10 m x 0.25 mm i.d., with 0.25 μm liquid film thickness. Operating conditions were: column temperature, 200°C; injector temperature 230°C; detector temperature, 240°C; and carrier gas hydrogen at 10 psi. Individual peaks were identified by comparing retention times with standard FAME. Peak areas were integrated and expressed as percentage of total FAME.

3.5 Yield and Composition of the Oil as Affected by Steam and/or Dry Heat Treatment

3.5.1 FAME analysis

Whole and crushed seed samples were treated with dry heat (30 min at 100°C) or with steam in the autoclave unit (15 and 30 min at 100°C), followed by 15 min drying at 100°C, as shown on Figure 3.2. Lipid extraction was achieved

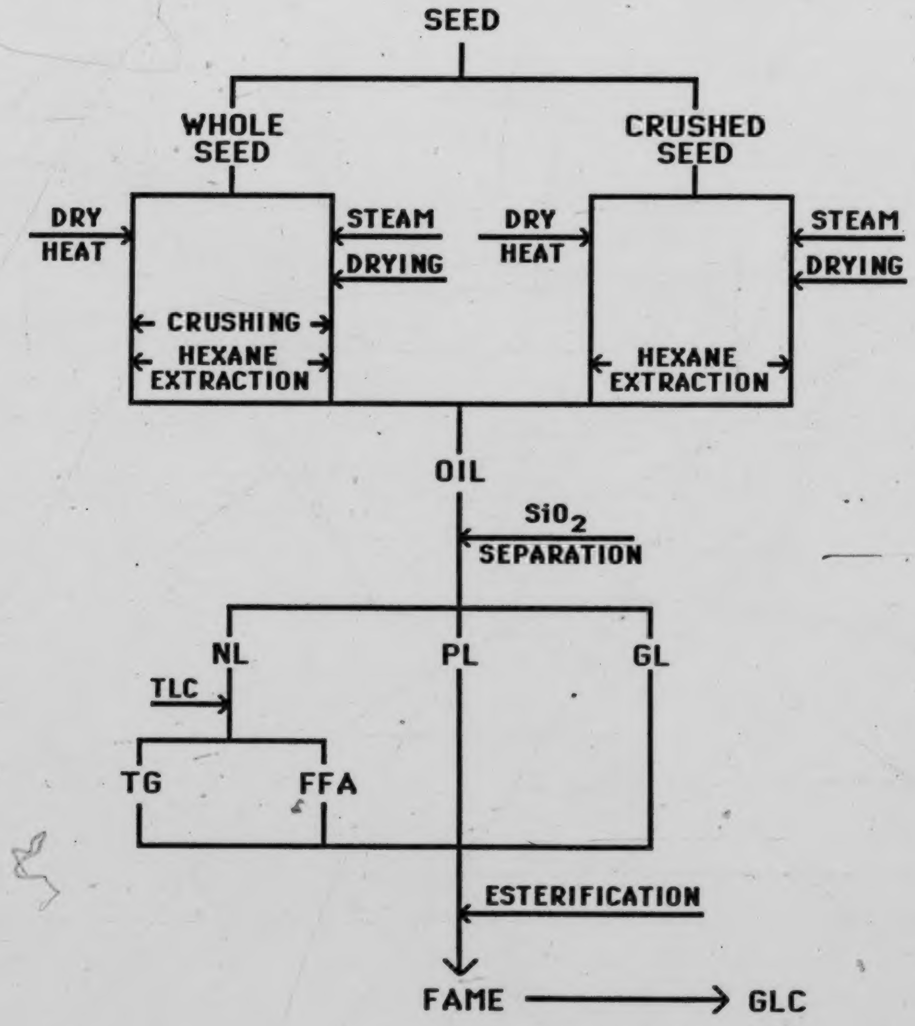

SEED
WHOLE SEED
CRUSHED SEED
DRY HEAT
STEAM
DRYING
CRUSHING
HEXANE EXTRACTION
DRY HEAT
STEAM
DRYING
HEXANE EXTRACTION
OIL
SiO2
SEPARATION
NL
PL
GL
TLC
TG
FFA
ESTERIFICATION
FAME
GLC

Figure 3.2 Sample preparation for the analysis of effects of dry heat and steam treatment(s) on oil yield and composition.

by the Goldfish method, using n-hexane (b.p. 63-66°C) as a solvent. Extraction was carried out for 6 hr. The solvent was removed from the oil extract under nitrogen, and the oil residue was determined gravimetrically, then redissolved in chloroform and stored in the dark at -18°C. Subsequent lipid fractionation on SiO_2 column, preparative TLC separation, and interesterification of lipids and GLC analysis of FAME were performed as described above. Phospho- and glycolipid fractions were esterified directly by preparative TLC, without further separation into individual constituents.

3.5.2 Quantitative determination of phosphorus, sulfur, chlorophyll and carotenoids

3.5.2.1 Sample preparation

An attempt was made to simulate the actual processing conditions. Oil from heat-treated samples (Figure 3.3) was obtained by expelling with a laboratory hydraulic press at 20,000 psi and by extracting the residual cake with n-hexane as described above. Both expelled and extracted lipid samples were centrifuged at 18,000 rpm for 15 min, and the supernatant was evaporated to dryness under vacuum to obtain pure lipid residues for subsequent analysis.

3.5.2.2 Phosphorus analysis

The combustion method under compressed oxygen, described by Yuen and Kelly (1980), was applied. A lipid sample of 0.4 g was weighed into a capsule containing

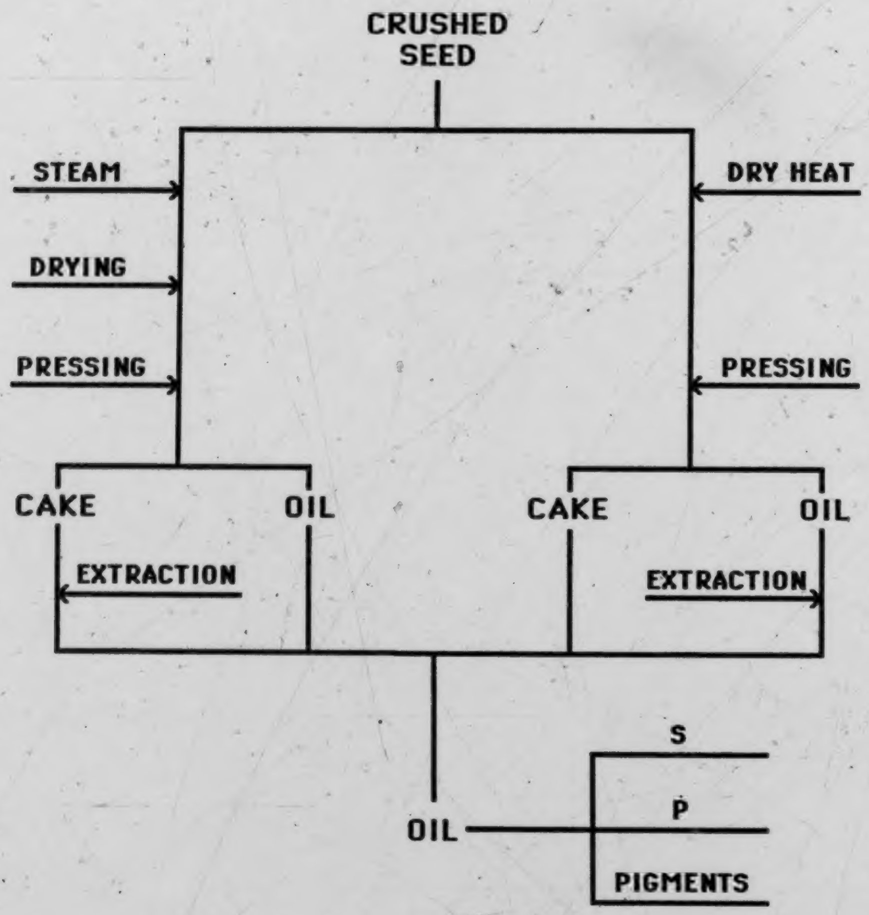

CRUSHED SEED
STEAM
DRYING
PRESSING
DRY HEAT
PRESSING
CAKE
OIL
CAKE
OIL
EXTRACTION
EXTRACTION
OIL
S
P
PIGMENTS

Figure 3.3 Sample preparation for the analysis of effects of dry heat and steam treatment(s) on sulfur (S), phosphorus (P), chlorophyll and carotenoid concentration in prepressed and extracted oils.

0.03-0.04 g ZnO, and heated on hot plate to disperse the catalyst in the oil. The capsule was then mounted on a loop holder and the nickel alloy fuse wire was fastened to the electrodes so that the loop was barely touching the surface of the sample in the capsule. The bomb was closed and filled with oxygen to 25 atm. The apparatus was transferred to a waster coolant, ignition wires were attached and the samples were combusted upon ignition. After 30 sec, to allow completion of the reaction, the pressure in the bomb was carefully released and the capsule was removed. The inner walls of the bomb, as well as the outer walls of the capsule, were rinsed with small portions of distilled H_2O into a 25 ml volumetric flask. The ashed sample in the capsule was dissolved in dilute nitric acid (HNO_3:H_2O, 1:10, v/v) and transferred to the same volumetric flask. The volume was made up to 25 ml with distilled H_2O. The content was filtered through a 0.45 μm pore size nylon filter. Aliquots of 10 ml of clear filtrate were transferred to a clean 25 ml volumetric flask and 5 ml of molybdo-vanadate coloring reagent was added. After 5 min to allow completion of the complexing reaction, the absorbance was read at 400 nm using a Beckman DU-8 scanning spectrophotometer. Phosphorus concentration was determined from a standard curve.

3.5.2.3 Sulfur analysis

The modified Johnson-Nishita (1952) method described by Tabatabai and Bremner (1970) was applied. Dry lipid sample (1 ml) was transferred into a microKjeldahl digestion flask and 3 ml sodium hypobromite solution was added. Flasks were occasionally swirled, then transferred after 5 min to the digestion rack at 250°C. After the content had evaporated, heating was continued for an additional 30 min, then the samples were cooled. The dry residue was resuspended by heating with 2 ml distilled H_2O. Then 4 ml reducing agent and 5 ml 1 N NaOH were added and the flasks were quickly attached to the digestion-distillation apparatus. Distillation was continued for 30 min, after which the distillate was reacted with 2.5 ml bismuth reagent. Absorbance readings were taken at 350 nm using a Bausch and Lomb Spectronic 20 spectrophotometer, and the sulfur concentration in ppm was calculated using a standard curve prepared under identical conditions.

3.5.2.4 Chlorophyll and carotenoid analysis

Lipid samples (1 ml) were dissolved in 10 ml dichloromethane and scanned from 350 to 750 nm using a Beckman DU-8 spectrophotometer. Peaks at 670 nm (chlorophyll) and 450 nm (β-carotene) were identified and the absorbance values were compared with standards. Chlorophyll content in ppm was read from the standard curve, while carotenoids were expressed as percentage of the largest observed peak for β-carotene.

Initial results suggested further experimentation with respect to the steaming time, as well as the temperature of the dry heat post-treatment. Steaming time was extended to 40 min, and the temperatures of subsequent drying were 105°C and 120°C (Table 3.1).

3.6 Glucosinolates in Canola Meal as Affected by Steam and/or Dry Heat Treatment

Standard thiourea UV assay (Wetter and Youngs, 1976) was applied for analysis of glucosinolates. Crushed seed samples (0.5 g) in 4 ml vials were steam or dry heated, or left at room temperature for 5-30 min (Figure 3.4). Lipids were extracted by filling the vials with n-pentane and leaving them capped at room temperature. After discarding the supernatant, samples were repeatedly rinsed with pure solvent to ensure that the meal was lipid-free. Samples were dried at 50°C under vacuum, cooled, and the weight was adjusted to less than, but close to 100 mg.

Phosphate-citrate buffer of pH 7 (0.5 ml) was added to each vial and the samples were heated in a Thermolyne block digestor for 5 min at 100°C to prevent formation of nitriles upon glucosinolate hydrolysis (McGregor, 1983). After cooling, 0.5 ml myrosinase solution (prepared by dissolving 400 mg of myrosinase in 90 ml phosphate-citrate buffer) and 2.5 ml of dichloromethane were added. One hour was allowed for phase separation and extraction of breakdown products.

A 100 μl aliquot of the clear dichloromethane layer was

Table 3.1 Summary of heat treatments of whole and/or crushed seeds for fatty acids, phosphorus, sulfur and pigment (chlorophyll and carotenoids) analysis.

Code				
	Dry heat treatment			
	Temperature (°C)		Time (min)	
A	100		30	
	Steam treatment			
			Drying	
	Temperature (°C)	Time (min)	Temperature (°C)	Time (min)
B	100	15	100	15
C	100	15	100	30
D	100	30	100	15
E	100	30	100	30
F	100	40	100	30
G	100	40	120	30

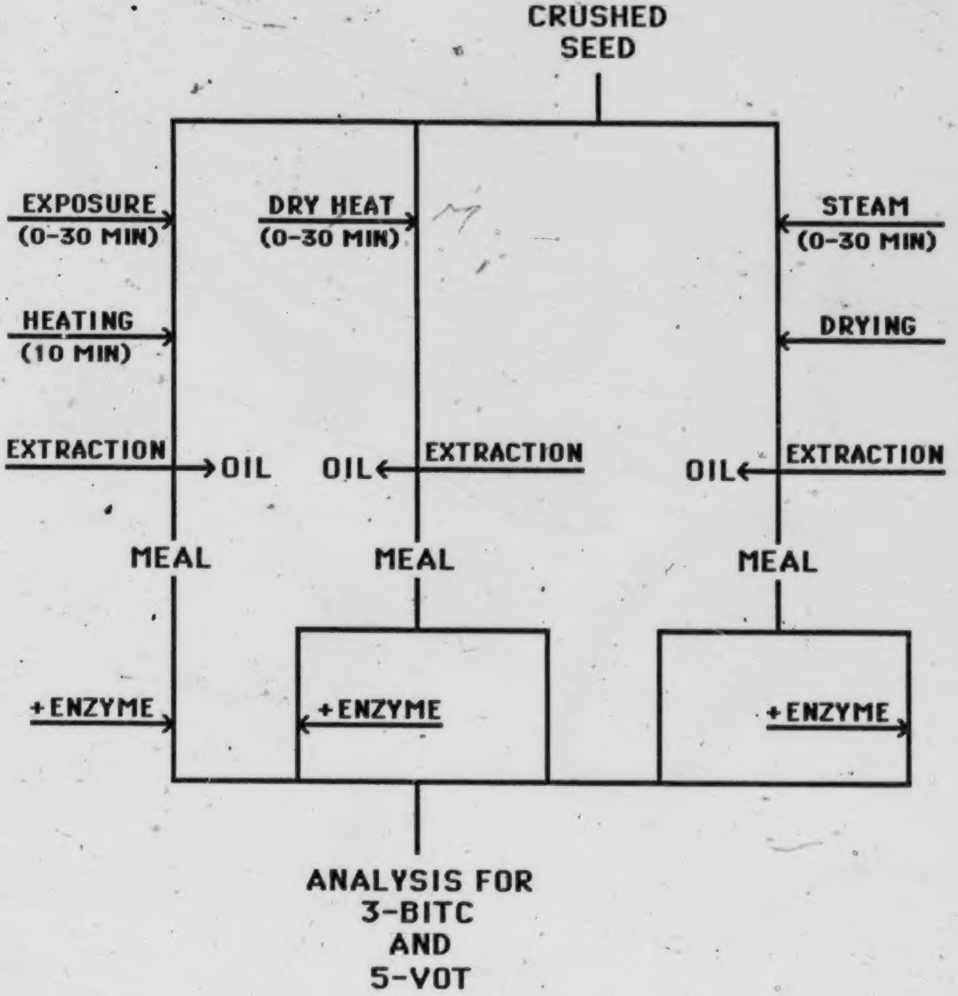

CRUSHED SEED
EXPOSURE (0-30 MIN)
HEATING (10 MIN)
EXTRACTION
OIL
MEAL
+ENZYME
DRY HEAT (0-30 MIN)
EXTRACTION
OIL
MEAL
+ENZYME
STEAM (0-30 MIN)
DRYING
EXTRACTION
OIL
MEAL
+ENZYME
ANALYSIS FOR 3-BITC AND 5-VOT

Figure 3.4 Sample preparation for the analysis of effects of holding time, dry heat and steam treatment on the concentration of 3-butenylisothiocyanate and 5-vinyloxazolidinethione.

transferred via syringe to a screw-capped 10 ml glass tube containing 6 ml 20% ammonia-95% ethanol (1:4, v/v) for conversion of alkenyl glucosinolates to their thiourea derivatives. For the analysis of hydroxy-alkenyl glucosinolates, 100 µl of the same sample was transferred to tubes with 6 ml of 95% ethanol.

Absorbance readings were taken at 235, 245 and 255 nm and the corrected optical density was calculated from the formula:

$$A_{corr} = A_{245} - (A_{235} + A_{255})/2$$

Concentration in mg/g was determined using the factors from the regression lines of the standard curves (Wetter and Youngs, 1976) and expressed as 3-butenylisothiocyanate and 5-vinyloxazolidinethione, representing alkenyl and hydroxy-alkenyl glucosinolates, respectively.

3.7 Efficiency of Chlorophyll and Carotenoid Removal From Canola Oil as Affected by Bleaching Agent Type and Concentration

Two different bleaching agents, naturally-active Brazilian clay and Tonsil (Na-activated clay), were tested for their bleaching efficiency on commercial canola oil samples. Water-degummed oil samples were dried over sodium sulfate, followed by heating at 100°C for 5 min. Bleaching agent at concentrations of 100 ppm - 2% was then added and the heating with stirring was continued for 15 min. After cooling, samples were filtered through a Whatman No. 1 filter paper and the oil was analyzed for chlorophyll and carotenoids as described above.

3.8 Electron Microscopy

3.8.1 Scanning electron microscopy

Air-dried seed samples were cut in halves and quarters (transverse sectioning) with a razor blade and treated for 10 min with a 1% solution of osmium tetroxide. Samples were then dried with aqueous ethanol of gradually increasing concentration (50%, 70%, 90% and 100%) and coated with a 20 nm layer of gold before the examination of hull cross-section and cell tissue from the cotyledon using a Cambridge Stereoscan model 250 SE-microscope operated at 20 kV accelerating voltage.

3.8.2 Transmission electron microscopy

Whole canola seed samples were fragmented and transferred into a vial containing 3% glutaraldehyde:3% formaldehyde (1:1, v/v) in phosphate buffer (pH 6.8, 0.025 M). Samples were left overnight under vacuum at 4°C. The fixative was then discarded and the seeds were washed several times with pure phosphate buffer, followed by treatment with 1% osmium tetroxide in phosphate buffer for 4 hr at 0°C (ice bath).

Samples were washed with cold water for 6 hr and left in 0.5% aqueous uranyl acetate at 4°C overnight. Aqueous ethanol gradient (50%, 70%, 90% and 100%) was used for drying, repeating the washing three times with absolute ethanol to ensure complete dehydration.

Embedding medium was prepared as follows: mixture A, containing 36.4 g NMA (Nadic methyl anhydride; essentially a methylated maleic adduct of phthalic anhydride) was added to mixture B, consisting of 17.3 g NMA and 22.7 g DDSA (dodecenyl succinic anhydride), with constant stirring and 3 ml tridimethylaminoethyl phenol (DMP-30) catalyst were gradually added.

Specimens were treated twice for 30 min with propylene oxide before embedding medium:propylene oxide (1:1, v/v) was added, and the samples were left overnight to allow evaporation of propylene oxide.

Samples were then transferred to a rubber mold and a fresh embedding medium was added. Polymerization was carried at 60°C for 16 hr. Sections of 60-90 nm were cut using a Reichert-Jung Ultracut microtome equipped with a glass knife.

Staining was performed with 5% uranyl acetate in methanol for 10 min, and in 5% lead citrate in 0.01 N NaOH for 5 min, before examining the cross-sections of cotyledon and meristematic tissue. A Philips EM 300 electron microscope operated at 60 kV accelerating voltage was used.

4. RESULTS AND DISCUSSION

4.1 SEM and TEM analysis of canola seeds

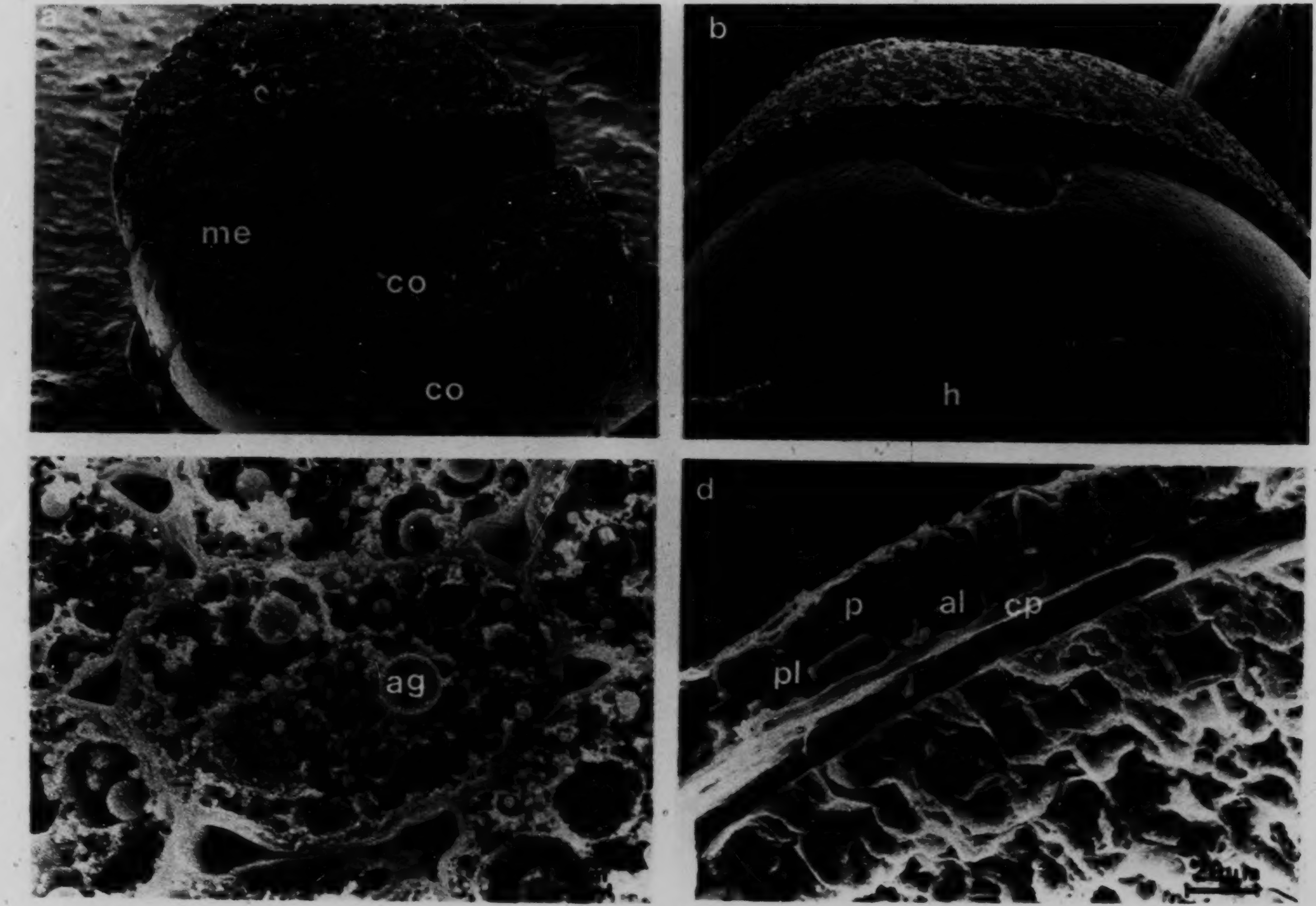
4.1.1 SEM analysis

The longitudinal cross-section of canola seed is shown on Figure 4.1a. Central meristem can clearly be seen in contrast to a conduplicate cotyledon tissue and it appears to be separated from the latter by a void space. This may be the result of dehydration and consequent shrinkage of both cotyledon and central meristem. Such shrinkage can also be seen on Figure 10b, which displays a partly-dehulled seed with the exposed surface of the outer cotyledon layer and inclusion of meristematic tissue.

The cross-sectional structure of the seed coat (hull) is shown in Figure 4.1d. It is comparable to the graphical representation of the detailed rapeseed microstructure presented on Figure 4.2. Easily recognized are the lignified cell walls in the palisade layer covered by less obvious, but still visible epidermis and subepidermis layers. Seed endosperm consists only of aleurone and crushed parenchyma monolayer cells; protein bodies can be seen embedded in the aleurone layer.

The SE-micrograph on Figure 4.1c reveals the structure of a single cell from the cotyledon tissue. While Wischnitzer (1981) regarded osmium tetroxide (OsO_4) as universal fixating agent, Arnott and Webb (1983) suggested that aqueous fixation of the oilseed material should include

Figure 4.1 SE-micrographs of canola seed. a) longitudinal cross-section of the seed; b) cotyledon surface; c) cross-section of hull; d) cell from cotyledon tissue. (me - meristematic tissue; co - cotyledon; ag - aleurone grains; h - hull; p - palisade layer; pl - pigment layer; al -aleurone layer; cp - crushed parenchyma).


a
c
me
co
co
b
h
ag
d
pl
p
al
cp

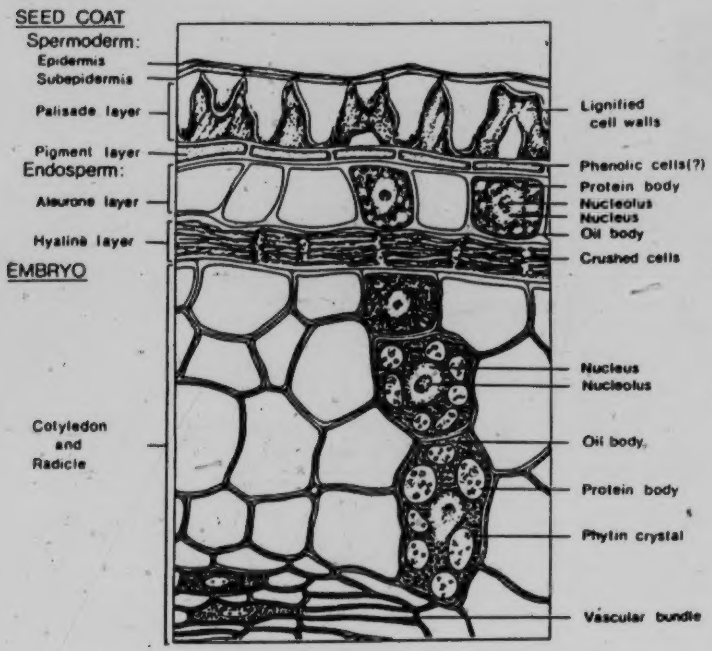

SEED COAT
Spermoderm:
Epidermis
Subepidermis
Palisade layer
Pigment layer
Endosperm:
Aleurone layer
Hyaline layer
EMBRYO
Cotyledon and Radicle
Lignified cell walls
Phenolic cells(?)
Protein body
Nucleolus
Nucleus
Oil body
Crushed cells
Nucleus
Nucleolus
Oil body
Protein body
Phytin crystal
Vascular bundle

Figure 4.2 Rapeseed microstructure (after Yiu *et al.*, 1983).

both glutaraldehyde and OsO_4 treatments for proper fixation of proteins and lipid bodies, respectively.

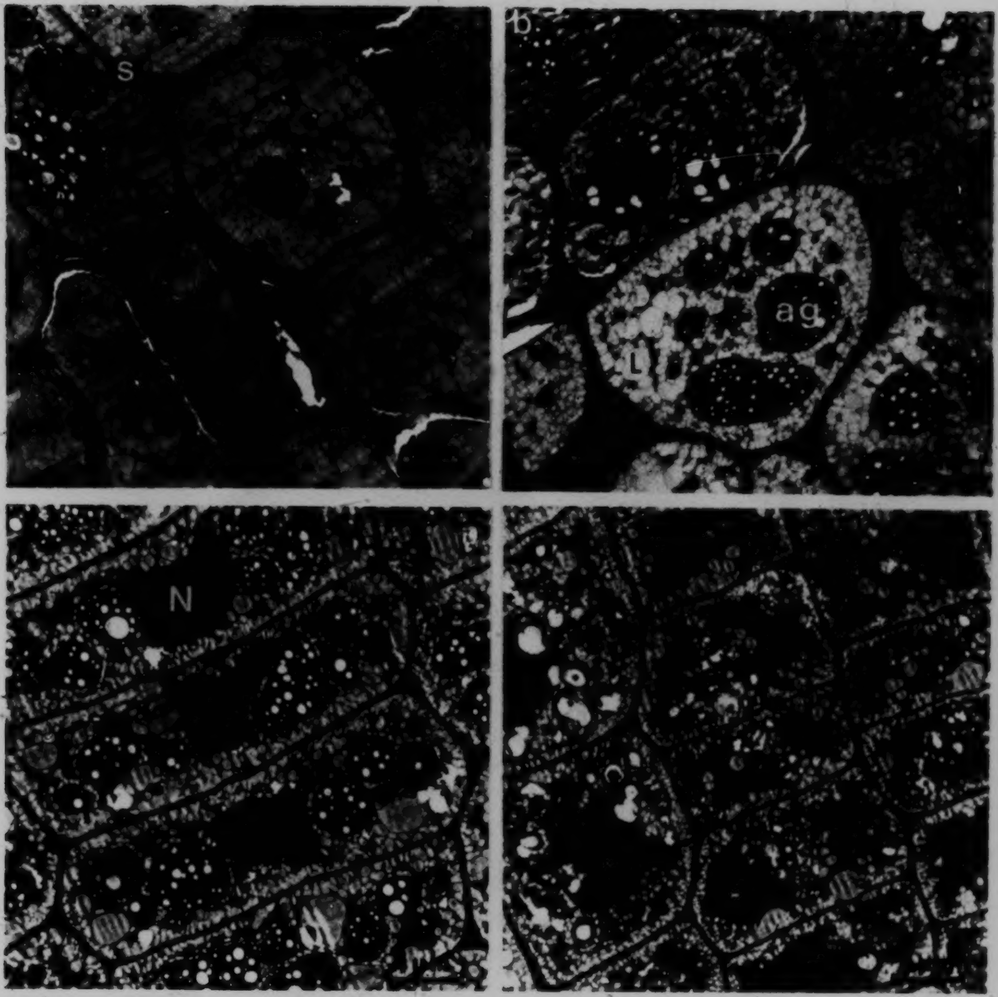
In our experiment, samples were treated only with buffered 1% OsO_4 solution which, nevertheless, gave satisfactory results. However, in some parts of conduplicate cotyledon (not shown here), the cell microstructure was not recognizable, probably due to insufficient protein fixation.

While the SEM method does not reveal the precise distribution of lipid bodies within the cell, as does TEM (Figure 4.3), the shape and size of protein bodies are better presented by SEM. Small, round bodies embedded in the aleurone grains are globoids (also referred to as phytin crystals) which consist mainly of Ca- and Mg-salts of phytic acid. Detailed study of globoids would be of interest since von Hofsten (1970) proposed, after analyzing 10^{-6} magnifications of a rapeseed cell, that globoids are surrounded by a double membrane of unknown structure.

4.1.2 TEM analysis

TE-micrographs of cell aggregates from the outermost cotyledon, inner cotyledon and central meristem are shown on Figure 4.3. The deformed, almost collapsed cells from the outer cotyledon were relatively poor in lipid bodies and had somewhat thinner cell walls as compared to neighboring cells of the inner cotyledon with thick cell walls, clearly seen middle lamellae and a large number of intercellular spaces. The cells from the central meristem were more rectangular in shape, most with a clearly seen nucleus in the middle; the

Figure 4.3 TE-micrographs of embryonal tissue cells. a) and b) cotyledon cells; c) and d) cells from central meristem. (ag - aleurone grains; cw - cell wall; L - lipid bodies; S - osmium artefacts).


s
b
ag
L
N

cell walls were thinner and there were fewer intercellular spaces. In some cells of the central meristem, no aleurone grains could be found. This might be a sign of the germination process in the seed. During germination, the protein becomes dissolved *in situ* and the protein bodies are replaced by vacuoles which may fuse into one large vacuole (Lott *et al.*, 1973).

Lipid bodies are not equally distributed between the cells. It appears that the cells from the inner cotyledon were on the average richest in lipid bodies. Data on cell wall thicknesses, as well as on the sizes of lipid bodies, aleurone grains and globoids are presented in Table 4.1. The smallest variation was observed in the diameters (D) of lipid bodies: >90% of lipid bodies were in the range 0.3-0.7 µm. Those globules with D>1.4 µm probably arose from the coalescence of two or more smaller lipid bodies. It appears that the lipid bodies distributed more densely along the plasmalemma and around the aleurone grains than in the rest of the cytoplasm.

The protein bodies were the most variable in size. Stanley *et al.* (1976), in their study of rapeseed microstructure by light microscopy, attempted to locate a major alkali-soluble rapeseed protein, i.e. 12 S glycoprotein. This was possible because 12 S glycoproteins are Schiff-positive and can be visualized under the microscope. Although the authors found that certain cells appeared richer in Schiff-reactive protein, these were randomly distributed throughout the seed.

Table 4.1 Size distribution of lipid bodies, aleurone grains and globoids in cells from rapeseed embryonal tissue'.

	Outer Cotyledon	Inner Cotyledon	Meristem
Lipid bodies			
0.3-0.7 µm	92	90	90
0.7-1.4 µm	7.5	7	9
>1.4 µm	0.5	3	1
Aleurone grains			
1-2 µm	98	64	--
2-3 µm	2	17	70
>3 µm	--	19	30
Globoids			
0.3-0.7 µm	100	91	86
0.7-1.4 µm	--	9	13
>1.4 µm	--	--	1
Cell wall thickness (µm)	0.26	0.47	0.15

' Diametric measurements of at least 50 cell constituents.

Artefacts resembling aleurone grains (Figure 4.3a) are the remains of OsO_4, the excess of which had not been properly removed by washing. The position of these dark spots also proves the preference of OsO_4 for interaction with lipids rather than with protein bodies. The differing contrasts of lipid bodies is also a sign of unequal osmiophily of the lipid material, since osmium preferentially reacts with the unsaturated lipids (Esau, 1977).

As can be seen from Figure 4.3a,b and c, globoids (dark spots embedded in aleurone grains) are distributed irregularly; their sizes, however, were quite uniform and ranged between 0.3 and 0.7 µm. White spots on protein bodies are "holes" from which the globoids were displaced upon sectioning the sample on the microtome cutter.

The appearance and the thicknesses of cell walls of cotyledon and meristematic cells are characteristic and can be used in distinguishing the two tissues. Cell walls from the inner cotyledon are thick (about 5 µm) and not as electron dense as the cell walls from the central meristem; the latter cell walls are also relatively thin (0.15 µm) due to the uncompleted cell differentiation. The shape and the density of meristematic cells obviously permits a smaller number of intercellular spaces as compared to the cotyledon cells. These intercellular spaces are considered to be important for translocation of nutrients and metabolic products within the seed (Esau, 1977). Most commonly, intercellular spaces develop by separation of contiguous cell walls through the middle lamella. The process starts in the corner where more than two cells are joined, and spreads to other wall parts. This type of intercellular space is known as schizogenous. Another type results from the breakdown of entire cells and is called lysigenous (Esau, 1977). However, electron microscopic studies by Fagerlind *et al.* (1974) indicated that the formation of intercellular spaces is a complex phenomenon, since an accumulation of membraneous structures may precede the development of cavities between the cells.

It appears that in some of the cells a dislocation of the protoplasmic material had occurred, while the cell wall remained physically unchanged.

Higher magnification of lipid bodies would be required in order to establish precisely their status in the cytoplasm. Von Hofsten (1970) found that some of these bodies were surrounded by a membrane, which matches the description of spherosomes. However, Esau (1977) advocated the combined view of the form of lipid bodies, according to which they exist in the form of both spherosomes, membrane-bound organelles in which hydrolases are associated with triacylglycerols (Matile, 1974), and membrane-free droplets. The latter form suggests that these droplets, being surrounded by cytoplasm biofluid, that is an aqueous system, exist in the form of semisolid mesomorphic hexagonal I structures.

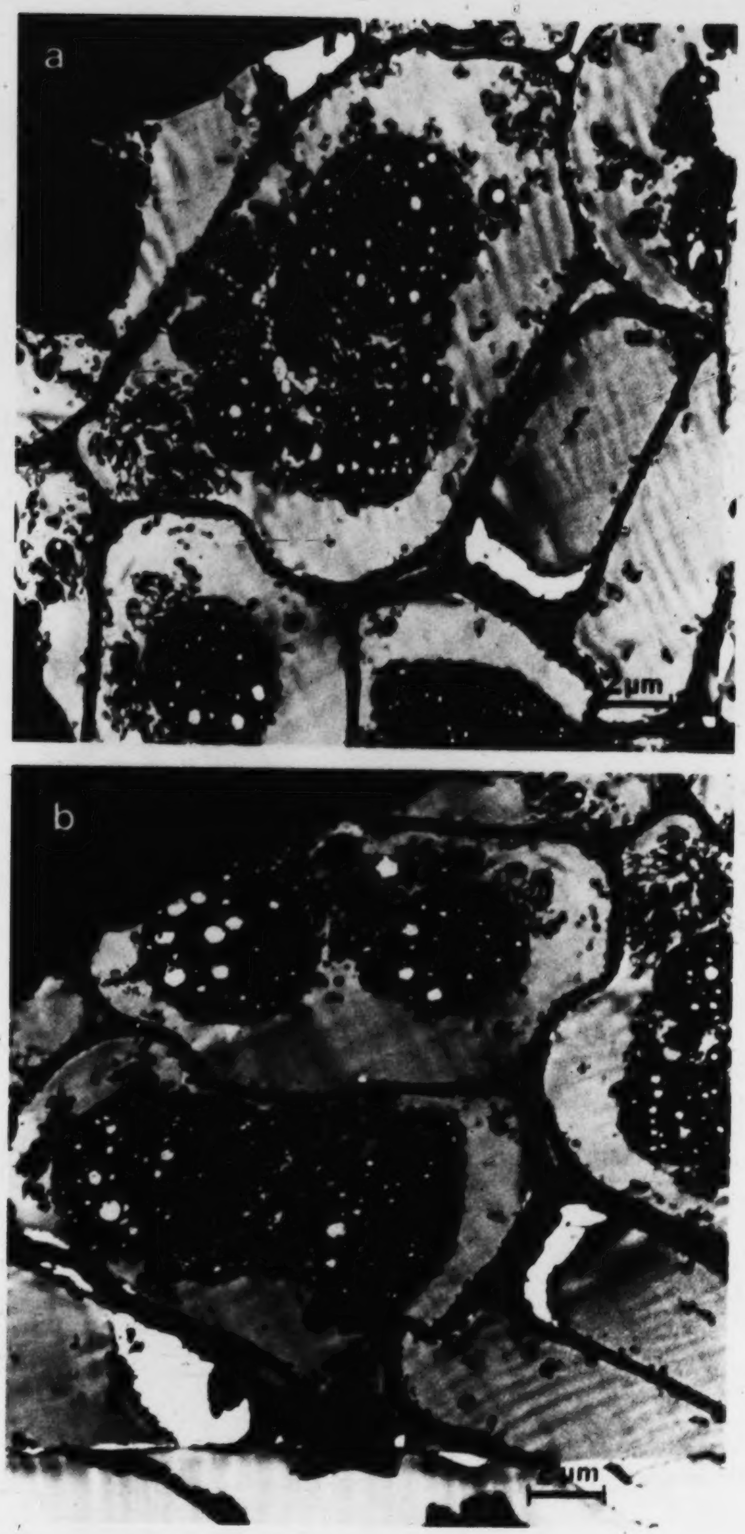
Treatment of seeds with increasing concentrations of aqueous ethanol prior to fixation with OsO_4 resulted in the extraction of lipid bodies (Figure 4.4). The remaining lipid globules were concentrated between the aleurone grains and were retained probably due to the physical inability of the solvent to penetrate into the spaces between densely concentrated aleurone grains.

Yiu *et al.* (1983) examined the effects of processing on seed tissue and on cell structure by using a fluorescence microscopic technique. They found that mechanical crushing had caused transversal separation of cell walls. Cooking and expeller pressing affected both proteins and lipids, as the former fused to form large aggregates encompassing phytin-containing globoids, while the latter coalesced into irregularly-shaped large droplets. The structural and microchemical organization of the hull was not affected by processing. Solvent extraction removed, according to the authors, most of the lipids since they were absent from the cotyledon cells after desolventization. The cotyledon fragments of the residual meal were composed of amorphous protein matrix filled irregularly with globoid inclusions and supported by a network of broken cell walls.

4.2 Lipase and Lipoxygenase Activity of Crushed Canola Seeds

Effects of lipase and lipoxygenase (LOX) activity in the crushed seeds as a function of time were monitored through gravimetric determinations of lipid fractions separated on silicic acid column and expressed as percentage of the seed total lipids (Table 4.1). Furthermore, variations in fatty acid composition in free fatty acids

Figure 4.4 TE-micrograph of cotyledon cells after dehydration of seed with increasing concentrations of aqueous ethanol (50%, 70%, 90%, 100%) and postfixation with 1% OsO_4.


a
2μm
b
2μm

(FFA), triacylglycerols (TG), glycolipids (GL), phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) were used as an indicator of enzymatic activity. Since the lipase and LOX enzymes come into contact with lipids from ruptured cells upon crushing of the seed, lipolysis and oxidative degradation of fatty acids may occur, resulting in the increase in NL fraction (TG + FFA) coupled with a decrease in the PL and GL fractions, changing the fatty acid composition in all fractions.

However, as seen from the Tables 4.2 and 4.3, there was no significant difference in oil composition as affected by the holding time of 5-30 min. The same was true for the fatty acid patterns in six lipid fractions (Figures 4.5-4.10); the greatest stability was found in triacylglycerols (Figure 4.6).

Consequently, no significant correlation could be established between holding time and lipase and LOX activities in canola seeds during crushing. The absence of lipolytic activity in this case might be related to a low moisture content of the seeds (5.2%) and to insufficient holding time (maximum 30 min).

Cuendet *et al.* (1954) observed lipolysis in wheat flour at moisture levels as low as 3%, but only after 10 weeks of storage. Furthermore, neutral pH, at which the experiment had been carried out, could also contribute to the absence of lipolytic activity. Wetter (1956) found rapeseed (*B. campestris*) lipase to have a sharp pH optimum at 8.5. At pH 5 enzyme activity was not evident at all, and fell rapidly

Table 4.2 Effect of holding time on the lipid composition in the seed.

	Time after crushing[1] (min)				
	0	5	10	15	30
	Amount applied (mg)				
	152.0	158.3	148.5	138.8	153.8
Neutral lipids[2] (mg)	134.0	147.0	133.4	125.5	132.6
Neutral lipids (% total)	95.2±0.4	94.7±0.6	94.7±0.1	95.2±0.7	94.6±0.6
Glycolipids[2] (mg)	1.7	1.7	2.97	1.6	2.0
Glycolipids (% total)	1.2±0.1	1.1±0.0	2.1±0.9	1.2±0.3	1.4±0.2
Phospholipids[2] (mg)	5.0	6.5	4.5	4.7	5.6
Phospholipids (% total)	3.5±0.1	4.2±0.3	3.2±0.3	3.6±0.5	4.0±0.7

[1] At room temperature.

[2] Average of 5 replicates.

Table 4.3 Summary of variance analysis.

	Level of Difference			
	5%	1%	5%	1%
Factor	Oil yield		Oil composition	
Treatment (A, B or D')	N.S.	N.S.	N.S.	N.S.
Seed condition whole/crushed	N.S.	N.S.	N.S.	N.S.
Holding time	--	--	N.S.	N.S.
Replication (5)	N.S.	N.S.	N.S.	N.S.

'See Table 3.1 for code explanation.

Table 4.4 Summary of Duncan's Multiple Range Analysis.

Factor	Oil	Level of Difference 5%	 1%
Phosphorus	Pressed	A B C D G E F	A B C D G E F
	Extracted	A B D G F $\underline{\text{C E}}$	A B D G F $\underline{\text{C E}}$
Sulfur	Pressed	A $\underline{\text{B C}}$ D F E G	A $\underline{\text{B C}}$ D F E G
	Extracted	A B D C F E G	A B D C F E G
Chlorophyll	Pressed	$\underline{\text{G A F}}$ $\underline{\text{E C}}$ B D	$\underline{\text{G A F}}$ $\underline{\text{E C}}$ B D
	Extracted	A E G $\underline{\text{B C}}$ F D	A $\underline{\text{E G}}$ $\underline{\text{B C}}$ F D
Carotenoids	Pressed	A $\underline{\text{G C}}$ $\underline{\text{B E}}$ F D	A $\underline{\text{G C}}$ $\underline{\text{B E}}$ F D
	Extracted	A C G B E F D	A C $\underline{\text{G B}}$ E F D

[1] Letters A-G correspond to treatment codes in Table 3.1 and represent means of 5 replicates. A line typed under any sequence of means indicates no significant difference at the given percent level.

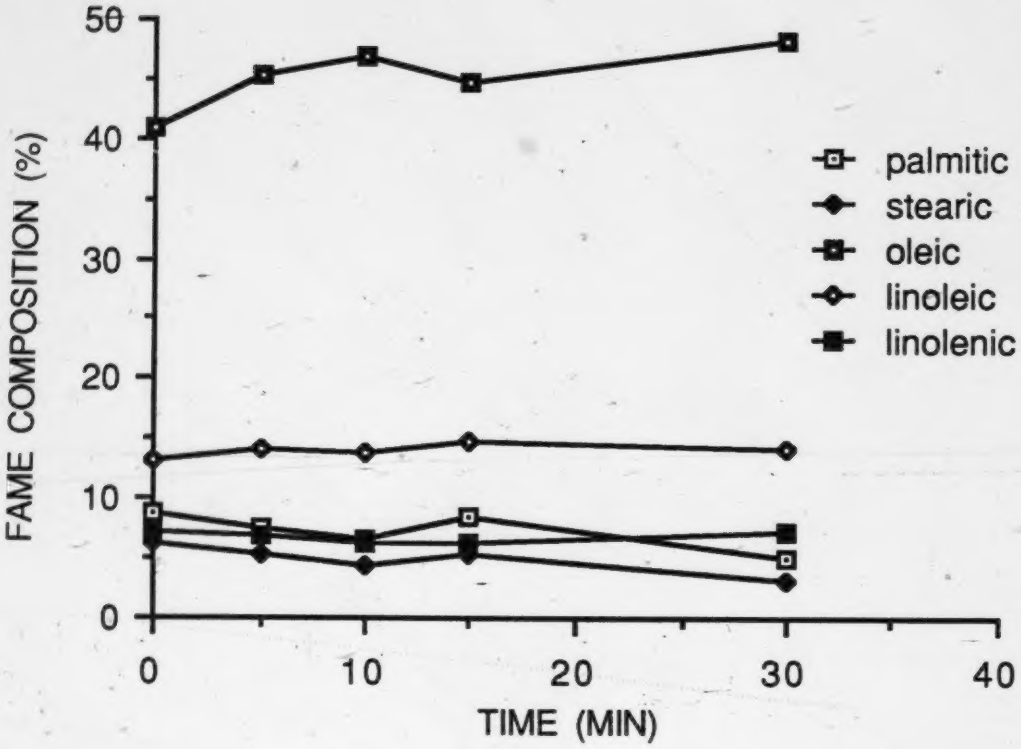


Figure 4.5 Fatty acid composition of free fatty acid fraction.

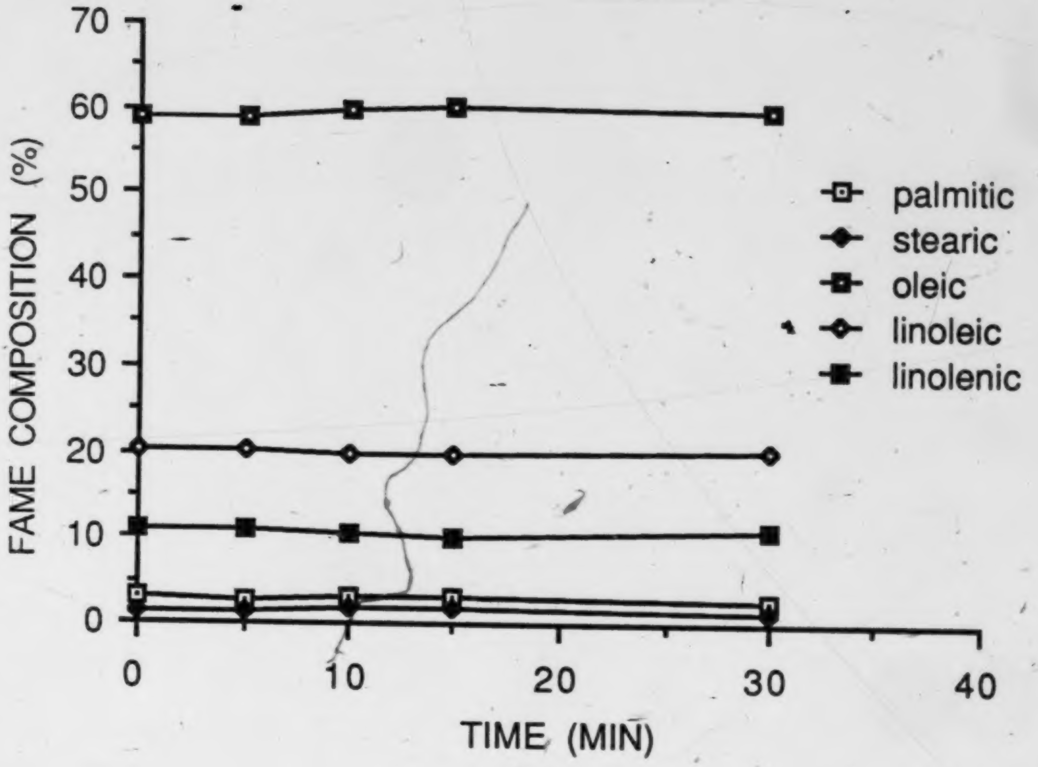


Figure 4.6 Fatty acid composition of triacylgycerol acid fraction.

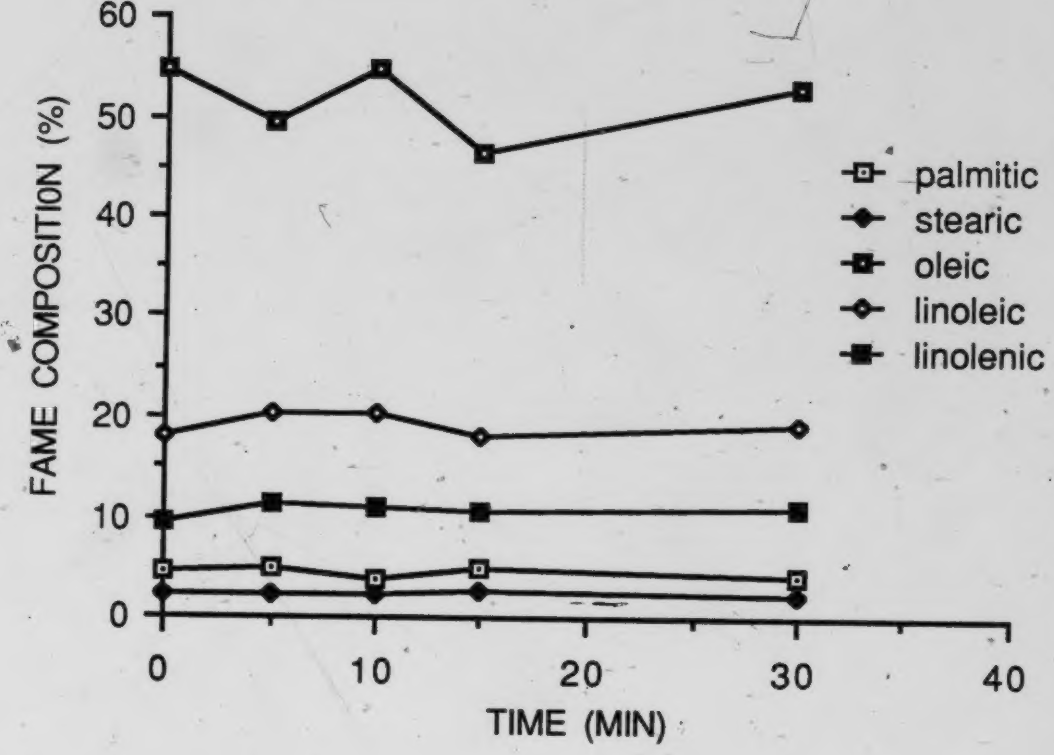


Figure 4.7 Fatty acid composition of glycolipid fraction.

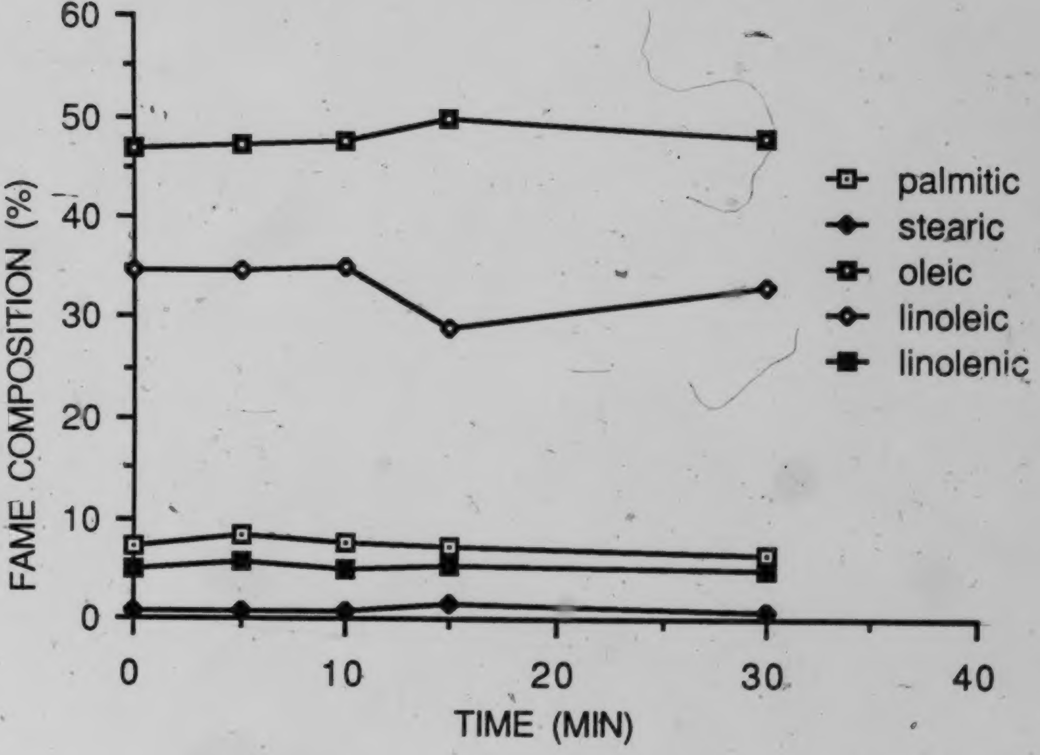


Figure 4.8 Fatty acid composition of phosphatidylcholine fraction.

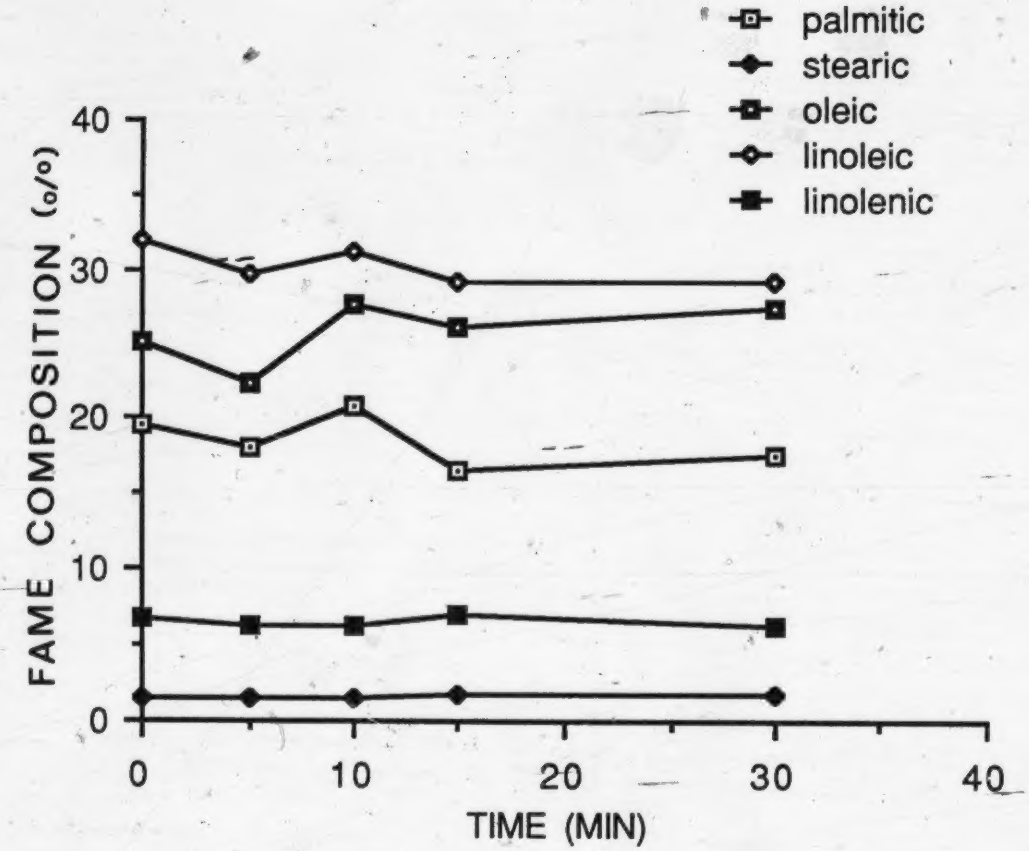


Figure 4.9 Fatty acid composition of phosphatidylinositol fraction.

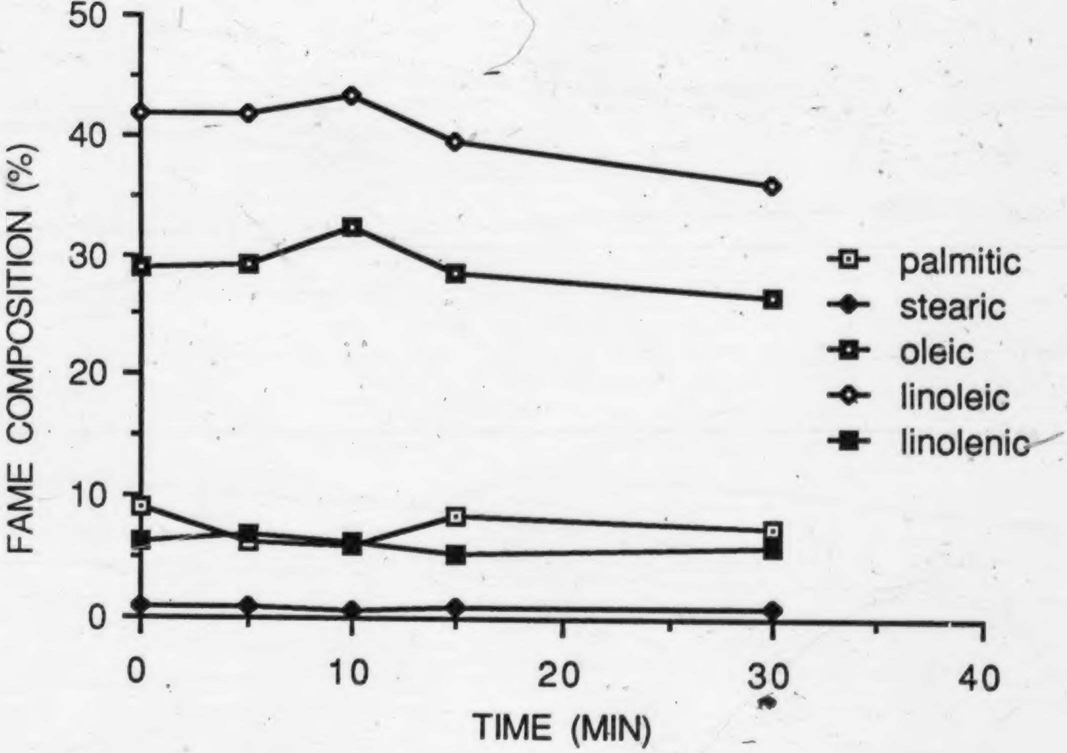


Figure 4.10 Fatty acid composition of phosphatidylethanolamine fraction.

at higher alkaline pH.

An explanation for the absence of LOX activity in rapeseed was proposed by St. Angelo *et al.* (1984). They showed that, in cultivars with erucic acid concentration as low as 0.2%, LOX activity was completely inhibited.

The fatty acid pattern in triacylglycerols, as found in our experiment (Figure 4.6), closely resembles the total fatty acid composition in canola oil (Ackman, 1983).

Somewhat higher proportions of $C_{16:0}$ and $C_{22:1}$ in the free fatty acid fraction are probably due to the preference of lipase for palmitic and erucic acid during the increased enzymatic activity in the germinating seed (Tookey *et al.*, 1964).

4.3 Effects of Heat Treatment on Quality of Canola Oil and Meal

4.3.1 Oil yield, composition and fatty acid pattern of oil fractions

An towards an increase in yield of phospholipids was observed in samples treated 15 and 30 min with steam, as compared with the results for dry heat treatment (Table 4.5). The steam is involved in splitting lipoprotein linkages, which results in a higher yield of phospholipids upon seed pressing and solvent extraction (Ansell, 1969).

The proportion of neutral lipids and glycolipids remained unchanged. No significant difference arose in the composition of lipids from whole and crushed seeds; the oil

Table 4.2 Yield and composition of oil from dry heat and steam-treated seed.

Treatment	Seed	Oil yield[1] (%)	Neutral Lipids		Glycolipids		Phospholipids		Amount applied (mg)	Recovery (%)
			(mg)	(% total)	(mg)	(% total)	(mg)	(% total)		
Dry heat 100°C, 30 min	W[2]	40.7±0.9	142.2	98.4±0.6	1.1	0.8±0.1	1.2	0.8±0.0	154.5	93.5±0.8
	C[3]	41.4±1.1	154.0	98.3±0.9	1.4	0.9±0.0	1.3	0.8±0.1	180.7	86.7±0.5
Steam 100°C 15 min; drying 100°C, 15 min.	W	42.0±1.2	153.3	97.7±0.4	1.5	0.9±0.0	2.1	1.3±0.2	178.6	87.8±0.9
	C	41.9±1.5	138.4	97.5±0.0	1.2	0.8±0.2	2.5	1.7±0.3	155.5	91.4±1.1
Steam 100°C 30 min; drying 100°C, 15 min	W	42.2±0.7	139.3	97.9±0.6	1.3	0.9±0.1	1.7	1.2±0.1	156.3	91.0±1.3
	C	42.3±1.1	132.1	97.8±0.2	1.3	0.9±0.0	1.7	1.3±0.2	155.6	86.8±1.4

[1] On a dry matter basis; average of 5 replicates.

[2] Whole.

[3] Crushed.

yield, however, was slightly higher in the crushed seeds. Fatty acid composition did not appear to be affected by the different heat treatments (Tables 4.6-4.9).

4.3.2 Phosphorus, sulfur, chlorophyll and carotenoids

Analyses were performed on the oil obtained by prepressing and solvent extraction (Figure 3.3) and varying steaming times were combined with different drying times and temperatures (Table 3.1).

Phosphorus concentration in both prepressed and solvent extracted oils from dry heated seeds was highly significantly lower than the corresponding concentrations for the rest of the treatments (Tables 4.9 and 4.10). Among the steam treatments, the one with 30 min steaming followed by 30 min drying at 100°C gave the highest phosphorus concentration. In all cases, hexane-extracted oils were highly significantly higher in phosphorus as compared to the samples obtained by prepressing at 20,000 psi, which is in agreement with the data from the literature (Persmark, 1972).

A pattern similar to that for phosphorus was found for sulfur (S) concentration in the oil. This supports the finding of Gronowitz *et al.* (1978) that the glucosinolates are unstable in aqueous medium. In addition, higher drying temperatures (>110°C) may cause chemical breakdown of glucosinolates, giving rise to sulfur-containing, oil-soluble products (Anjou, 1972). This may explain the highest sulfur concentrations in oils obtained by treatment

Table 4.6 Fatty acid percent composition[1] of the free fatty acid fraction as affected by heat treatment of the seed.

Fatty Acid[2]	Treatment					
	Dry heat 100°C, 30 min		Steam (100°C) 15 min		Steam (100°C) 30 min	
	W[3]	C[4]	W	C	W	C
16:0	7.1	7.2	7.0	8.9	7.6	8.2
16:1	0.5	1.0	0.8	0.8	0.9	0.7
18:0	4.8	5.5	5.1	6.2	5.3	6.3
18:1	49.3	44.6	47.6	48.7	46.5	44.8
18:2	15.7	15.6	13.2	13.4	16.5	17.4
18:3	5.9	5.6	4.9	4.7	6.2	6.4
20:0	1.1	1.2	1.0	1.2	1.1	1.0
20:1	2.1	1.9	1.7	1.8	2.2	1.8
22:0	0.9	1.1	1.2	1.1	0.9	1.0
22:1	2.2	1.7	2.5	1.9	1.6	1.8

[1] Concentrations given in this and following tables represent means of 3 replicates not varying more than 2% from the mean value.

[2] In this and the following tables, given as number of carbon atoms:number of double bonds.

[3] Whole seed.

[4] Crushed seed.

Table 4.7 Fatty acid percent composition of the triacylglycerol fraction as affected by heat treatment of the seed.

	Treatment					
	Dry heat 100°C, 30 min		Steam (100°C) 15 min		Steam (100°C) 30 min	
Fatty Acid	W	C	W	C	W	C
16:0	3.1	3.1	3.2	3.3	2.7	3.4
16:1	0.2	0.2	0.2	0.2	0.2	0.2
18:0	1.2	1.1	1.4	1.3	1.1	1.4
18:1	60.5	59.1	58.9	60.0	58.9	59.0
18:2	19.7	20.4	20.6	19.8	19.5	20.5
18:3	10.3	10.5	10.9	10.3	9.7	11.0
20:0	0.5	0.5	0.5	0.5	0.5	0.5
20:1	1.8	1.7	1.7	1.7	1.5	1.7
22:0	0.3	0.3	0.4	0.3	0.3	0.3
22:1	1.0	1.0	1.1	1.0	1.0	1.1

Table 4.8 Fatty acid percent composition of the phospholipid fraction as affected by heat treatment of the seed.

	Treatment					
			Steam (100°C)			
	Dry heat 100°C, 30 min		15 min		30 min	
Fatty Acid	W	C	W	C	W	C
16:0	11.4	10.8	10.4	9.5	8.7	8.6
16:1	1.2	0.9	0.8	0.9	0.6	0.8
18:0	1.6	1.5	1.7	1.5	1.4	1.6
18:1	40.0	37.8	39.5	40.4	38.3	42.1
18:2	30.4	34.3	29.4	30.3	30.1	29.6
18:3	6.5	6.0	6.4	6.3	5.8	6.8
20:0	0.5	0.9	0.5	0.7	0.7	0.4
20:1	0.4	0.7	0.4	0.5	0.4	0.5
22:0	0.3	0.6	0.3	0.4	0.5	0.2
22:1	0.4	0.4	0.6	0.5	0.3	0.5

Table 4.9 Fatty acid percent composition of the glycolipid fraction as affected by heat treatment of the seed.

	Treatment					
			Steam (100°C)			
	Dry heat 100°C, 30 min		15 min		30 min	
Fatty Acid	W	C	W	C	W	C
16:0	5.4	4.5	4.3	4.5	5.0	4.2
16:1	0.8	0.5	0.5	0.6	0.6	0.6
18:0	3.3	2.2	2.2	2.1	2.0	2.0
18:1	48.7	51.9	52.0	51.7	52.8	54.3
18:2	15.2	15.6	18.6	16.2	18.6	17.4
18:3	8.3	7.4	9.5	8.7	9.6	10.1
20:0	0.7	1.9	0.9	0.8	1.1	0.8
20:1	1.0	2.0	2.0	2.1	2.2	2.0
22:0	0.7	1.0	1.0	1.1	0.8	1.1
22:1	1.2	1.2	2.1	1.8	2.0	1.1

Table 4.10 Phosphorus (P) and sulfur (S) concentrations[1] in prepressed (PR) and extracted (E) oils from dry heat and steam-treated seed.

Text code designation	Treatment	Time (min)	Drying Time (min)	Drying Temp. (°C)	Oil	P (ppm)	S (ppm)
A	Dry heat 100°C	30	--	--	PR	30.2±1.2	1.4±0.2
					E	68.4±1.6	2.3±0.3
B	Steam 100°C	15	15	100	PR	104.3±2.3	3.8±0.3
					E	153.9±2.7	5.8±0.5
C	"	15	30	100	PR	115.0±2.2	4.0±0.1
					E	205.1±3.1	8.2±0.4
D	"	30	15	100	PR	126.3±1.7	5.4±0.5
					E	187.6±2.2	6.2±0.6
E	"	30	30	100	PR	146.0±2.3	7.8±0.3
					E	205.9±3.1	10.6±0.7
F	"	40	30	100	PR	152.6±1.7	6.4±0.3
					E	200.0±3.1	9.7±0.3
G	"	40	30	120	PR	137.2±1.5	10.6±0.5
					E	189.5±2.1	12.2±0.6

[1] Average of 3 replicates.

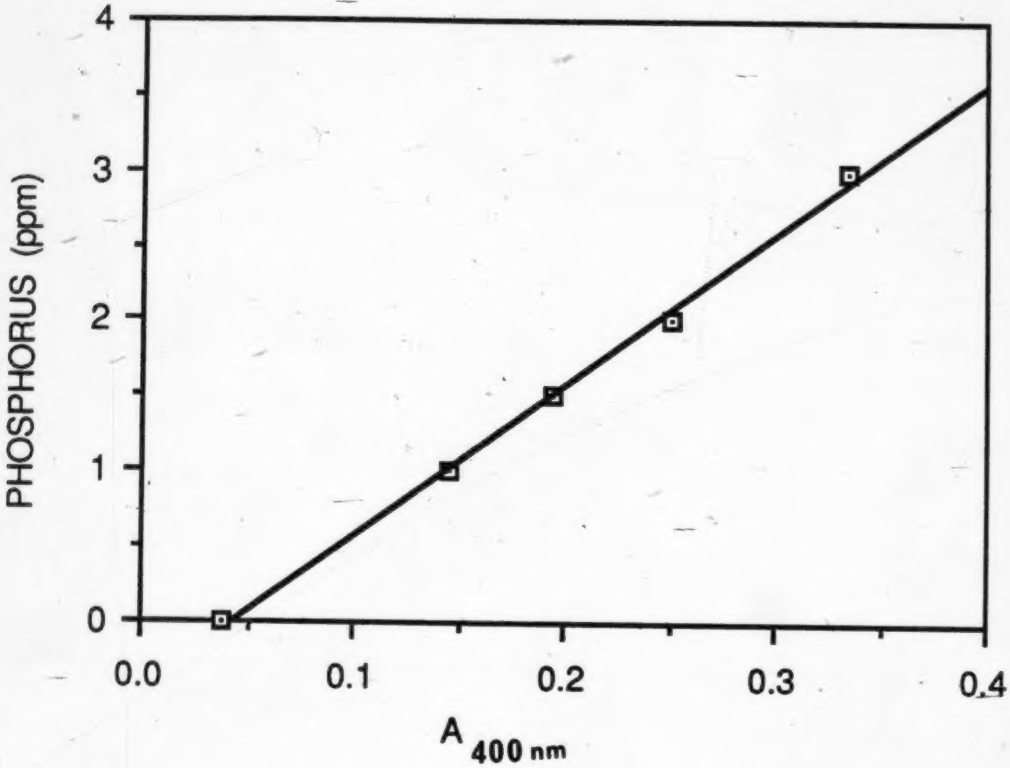


Figure 4.11 Calibration curve for phosphorus determination. Phosphorus was determined spectrophotometrically as a yellow molybdo-vanadate complex, after ashing samples in an oxygen bomb (Yuen and Kelly, 1980).

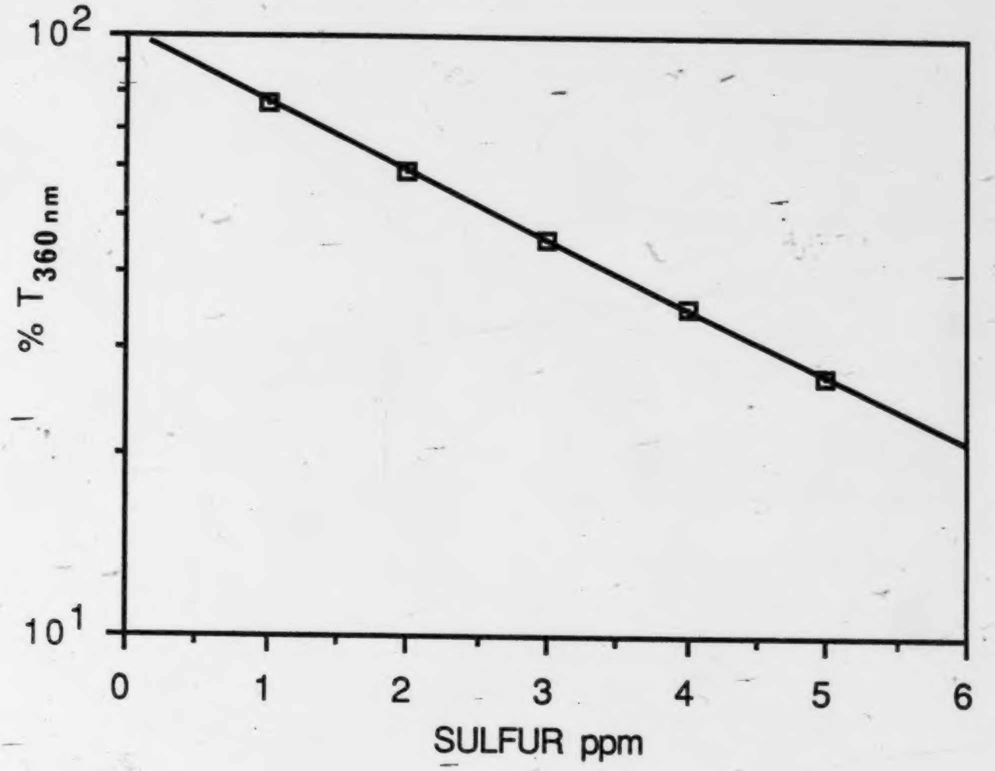


Figure 4.12 Calibration curve for sulfur determination. Sulfur was determined spectrophotometrically as a methylene blue complex with bismuth reagent, after wet digestion of samples in Na-hypobromite (Tabatabai and Bremner, 1970).

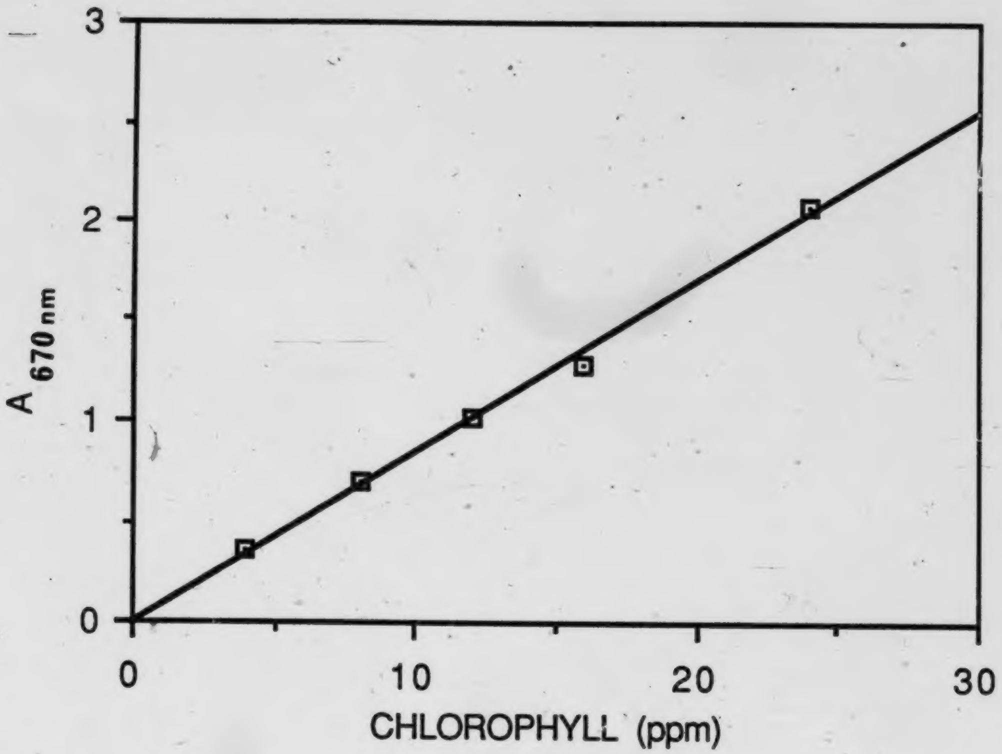


Figure 4.13 Calibration curve for chlorophyll determination. Chlorophyll was determined spectrophotometrically, after diluting 1 ml oil samples with 9 ml methylene chloride (AOCS method).

G. On an industrial level, this would require higher refining costs if low efficiency of hydrogenation is to be avoided (de Man, 1983; Klimmek, 1984). Vigorous conditions of oil expelling on an industrial scale cannot be well simulated on the laboratory press. Therefore, all of the pressed oils were lower in phosphorus and in sulfur concentration as compared to the solvent extracted oils.

Goss (1947) reported that 30-40% of the crude, nonrefined phospholipid fraction from industrial processing was triacylglycerols. The inclusion of neutral lipids in the precipitated lecithin sludge was explained by Desnuelle *et al.* (1952). They assumed that so-called mixed double-layers were formed on the surface of the water droplets in contact with the crude oil. These double-layers consist of neutral acylglycerols and phosphatides with the maximal stability of mixed double-layers at a molar ratio 1:2 between the neutral acylglycerols and phosphatides. This may explain the inclusion of about one-third neutral oil into the lecithin sludge.

Increased levels of phospholipids in oils from crushed steamed seeds would then lead to higher losses in the neutral lipids. This loss, however, could be compensated for by the application of phospholipids as emulsifying agents, which at the present is not in practice in the food industry in North America.

The influence of solvent polarity on the extractability of phospholipids was demonstrated by McKillican *et al.* (1970). The chloroform:methanol (2:1, v/v) extract of rapeseed oil contained ten times the phosphorus content of oil extracted with n-hexane, calculated on a total oil yield basis. Even though the difference in phospholipid yield was also evident from our experiments (Tables 4.2 and 4.7), this ratio could not be established due to the unequal treatment of the samples subjected to extraction.

The highest concentrations of chlorophyll were in the oils from treatment D (30 min steaming, followed by 15 min drying at 100°C; Table 4.11). Chlorophyll molecules, embedded in the lamellae of the chloroplast grana, are closely associated with lipids, proteins and lipoproteins. They are held together by mutual attraction and by the affinity of each molecule's phytol chain for lipids and by the affinity of each molecule's hydrophobic planar porphyrin ring for proteins. Chlorophyll molecules may therefore be visualized as embedded between the protein and lipid layers with the carotenoid positioned alongside the phytol chain of the chlorophyll (Francis, 1985). This may explain the high correlation in our experiments between chlorophyll and carotenoid concentrations in the oil.

Vigorous processing conditions, such as prolonged heating at high temperatures, might cause chemical transformation of chlorophyll, i.e. replacement of a central Mg-atom in the porphyrin ring by a proton, forming olive-brown pheophytins. Chlorophyll levels in oils from treatment G are the lowest of all steam treatments, which may be due to the high post-treatment temperature (120°C) and, consequently, to breakdown of chlorophylls to

Table 4.11 Chlorophyll and carotenoid concentrations[1] in prepressed and extracted oils from dry heat and steam-treated seeds.

			Drying				
Text code designation	Treatment	Time (min)	Time (min)	Temp. (°C)	Oil	Chlorophyll (ppm)	Carotenoids (ppm)
A	Dry heat 100°C	30	--	--	PR	7.0±0.3	56.0±1.1
					E	10.4±0.2	64.0±1.2
B	Steam 100°C	15	15	100	PR	13.0±0.3	90.9±1.4
					E	13.5±0.3	76.1±0.9
C	"	15	30	100	PR	10.5±0.1	89.4±1.1
					E	13.5±0.2	74.2±1.0
D	"	30	15	100	PR	15.0±0.2	100
					E	17.2±0.3	84.6±1.2
E	"	30	30	100	PR	10.4±0.1	91.1±1.2
					E	11.5±0.2	80.8±1.1
F	"	40	30	100	PR	8.0±0.4	97.7±1.3
					E	14.5±0.2	83.3±1.1
G	"	40	30	120	PR	8.0±0.2	88.9±1.1
					E	12.0±0.3	75.2±1.1

[1] Average of 3 replicates.

[2] Highest concentration was found in prepressed oil from treatment D and the carotenoid concentration in oils from other treatments was expressed as % of this concentration.

pheophytins.

Steam treatment releases chlorophylls together with carotenoids from their bound form, thus increasing the extractability of the pigments (Anjou, 1972). Carotenoids, however, appear to be more heat resistant than the chlorophylls, as their concentration in the oil had not been affected by seed drying at 120°C for 30 min (treatment G).

According to Francis (1985), carotenoids are susceptible to oxidative degradation. The reactions are believed to be caused by free-radical formation in three distinct steps. Carotenoids are usually more stable in systems with high degrees of unsaturation, possibly because the lipid system itself accepts the free radicals more easily than carotenoids. In canola oil, where total unsaturates exceed 90%, carotenoids are believed to be relatively stable, as suggested by the results listed in Table 4.11.

4.3.3 Glucosinolates in canola meal as affected by the holding time and by the heat treatment of the crushed seeds

The following objectives were considered in designing the experiment for glucosinolate analysis:

- to measure the concentration of glucosinolates in the meal obtained from the seeds exposed to holding time of 0 to 30 min holding time after crushing.
- to examine the influence of dry heat and steam treatments, both applied for 5-30 min, on myrosinase activity and on respective concentrations of intact and

hydrolysed glucosinolates in the meal.

In the standard thiourea-UV assay for total glucosinolates in rapeseed meal, expressed as 3-butenyl-isothiocyanate (3-BITC and 5-vinyloxazolidinethione (5-VOT), myrosinase enzyme isolated from yellow mustard and dissolved in phosphate-citrate buffer at pH 7 is added to moisture-free, lipid-free meal in order to induce controlled hydrolysis of glucosinolates. The breakdown products, volatile isothiocyanates and nonvolatile hydroxyl-group-containing isothiocyanates, are then converted into corresponding thioureas and oxazolidinethiones, respectively, and quantitated by UV-spectroscopy. McGregor (1983) summarized the method as follows:

(1) Preparation of oil-free meal.

(2) Inactivation of factors that give rise to nitrile formation upon enzymatic hydrolysis of glucosinolates.

(3) Hydrolysis of glucosinolates by the addition of supplementary myrosinase activity of a yellow mustard myrosinase isolate and simultaneous extraction of the isothiocyanates into methylene chloride.

(4) Formation of thiourea derivatives of the isothiocyanates and cyclization of nonvolatile isothiocyanates into oxazolidinethiones.

(5) Quantitative measurement of the thiourea derivatives and/or oxazolidinethiones by UV-spectroscopy.

Volatile alkenyl-isothiocyanates are converted to their substituted thiourea derivatives by reaction with ammonia in ethanol. Those isothiocyanates, which contain a hydroxyl group in their side chain, cyclize in 95% ethanol to form oxazolidinethiones. Both thiourea derivatives and oxazolidinethiones absorb in the UV-region at 245 nm. They are quantitatively determined by measuring this absorbance and subtracting background absorbance using measurements taken at 235 nm and 255 nm.

In the standard method, seeds are saturated with moisture in order to maximize the efficiency of subsequent inactivation of myrosinase by heating the seeds at 100°C for 10 min. In our experiment, however, only control samples were analyzed in this way (treatment D). In all other cases, this step was omitted in order to evaluate the function of the seed's natural moisture in enzyme inactivation upon the heat treatment (Figure 3.4).

Both dry heated and steamed samples were analyzed for glucosinolate content, with and without addition of the myrosinase. This was done for two reasons: Firstly, as mentioned above, to observe the extent to which different heat treatments, carried out for different amounts of time, contribute to the indigenous myrosinase inactivation and to measure the levels of isothiocyanates and vinyloxazolidinethiones in the meal without prior addition of the enzyme; secondly, to assess the effect of heat treatments on the total content of glucosinolates in the meal. In this case, mustard myrosinase solution was added, ensuring that complete glucosinolate hydrolysis had taken place.

Since no significant correlation was found between duration of seed holding time and of both dry heat and steam treatment (Tables 4.12-4.14) with the concentration of total and autolyzed glucosinolates, the results were averaged out for better clarity in comparing the control on one side (treatment D), and holding-time exposed, dry heated and steamed samples (treatments C, A and B, respectively) on the other, in terms of total and autolyzed glucosinolates in the meal (Table 4.15).

The difference in concentration of 3-BITC between the meals from treatments C and control (D) is higher than the difference in the corresponding concentrations of 5-VOT. This means that the seed's natural moisture was sufficient for indigenous myrosinase to initiate the hydrolysis of glucosinolates, although at a very low rate. The difference in 3-BITC content between treatments C and D suggests that the hydrolysis had taken place before the enzyme was added and that the loss in alkenyl-isothiocyanates occurred due to their volatility. The difference in concentration between the nonvolatile oxazolidinethiones is less noticable, but enough to suggest that the seed's moisture (5.2%) was not sufficient to release the glucosinolates from their bound form upon heat treatment. This can also partly explain the aforementioned difference in 3-BITC concentrations.

Addition of myrosinase to the dry heated samples (treatment A) resulted in 3-BITC and 5-VOT concentrations similar to those from treatment C. This supports the hypothesis that glucosinolates splitting from their complexes with proteins and/or polar lipids occurs at low

Table 4.12 Effect of crushed seed holding time on concentration of 3-butenylisothiocyanate[1] (3-BITC) and 5-vinyloxazolidinethione[2] (5-VOT) in the meal[3].

Holding Time (min)	3-BITC (mg/g)	5-VOT (mg/g)
0	2.07±0.19	1.78±0.14
5	1.98±0.15	1.62±0.01
10	1.88±0.25	1.74±0.19
15	2.00±0.14	1.58±0.19
20	1.86±0.11	1.76±0.11
25	2.04±0.07	1.55±0.17
30	1.82±0.07	1.44±0.05

[1] In this and in following tables, as determined spectrophotometrically at 245 nm as thiourea derivatives.

[2] In this and in following tables, as determined spectrophotometrically at 245 nm.

[3] Correlation coefficients between holding time and 3-BITC concentrations were:
holding time with 3-BITC, r=-0.57, not significant at 5% level.
holding time with 5-VOT, r=-0.01, not significant at 5% level.

Table 4.13 Effect of dry heat treatment of crushed seed on concentration of 3-butenylisothiocyanate (3-BITC) and 5-vinyloxazolidinethione (5-VOT) in the meal[1].

	3-BITC (mg/g)		5-VOT (mg/g)	
	Myrosinase			
Time (min)	-	+	-	+
5	1.37±0.01	2.04±0.17	1.17±0.00	1.73±0.08
10	1.83±0.17	2.01±0.31	1.50±0.13	1.67±0.30
15	1.56±0.06	1.92±0.34	1.34±0.14	1.42±0.30
20	1.60±0.12	1.63±0.18	1.37±0.07	1.60±0.05
25	1.79±0.08	2.19±0.03	1.52±0.07	1.56±0.08
30	1.70±0.06	2.30±0.13	1.61±0.06	1.49±0.48

---

[1] Correlation coefficients between the treatment time and the glucosinolate concentrations were as follows:
Time with 3-BITC (no enzyme added): -0.50, not significant at 5% level.
Time with 3-BITC (enzyme added): -0.51, not significant at 5% level.
Time with 5-VOT (no enzyme added): -0.43, not significant at 5% level.
Time with 5-VOT (enzyme added): -0.53, not significant at 5% level.

Table 4.14 Effect of steam treatment of crushed seed on concentration of 3-butenylisothiocyanate (3-BITC) and 5-vinyloxazolidinethione (5-VOT) in the meal'.

	3-BITC (mg/g)		5-VOT (mg/g)	
	Myrosinase			
Time (min)	-	+	-	+
5	0.24±0.05	2.85±0.00	0.17±0.00	1.82±0.18
10	0.20±0.00	2.70±0.49	0.15±0.00	1.80±0.17
15	0.22±0.00	2.26±0.49	0.15±0.03	1.84±0.07
20	0.20±0.03	2.72±0.00	0.13±0.01	1.48±0.08
25	0.22±0.03	2.55±0.08	0.13±0.01	1.89±0.06
30	0.22±0.04	2.53±0.63	0.12±0.01	2.08±0.15

' Correlation coefficients between the treatment time and the glucosinolate concentrations were as follows:
Time with 3-BITC (no enzyme added): -0.63, not significant at 5% level.
Time with 3-BITC (enzyme added): -0.66, not significant at 5% level.
Time with 5-VOT (no enzyme added): -0.51, not significant at 5% level.
Time with 5-VOT (enzyme added): -0.47, not significant at 5% level.

Table 4.15 Average concentrations of 3-butenylisothiocyanate (3-BITC) and 5-vinyloxazolidinethione (5-VOT) in meals from holding-time exposed[1], dry heat[2] and steam-treated[3] seeds.

Text Code Designations	Treatment	Myrosinase	3-BITC (mg/g)	5-VOT (mg/g)
A	Dry heat 5-30 min	+ -	2.01±0.23 1.64±0.17	1.58±0.11 1.42±0.16
B	Steam 5-30 min	+ -	2.60±0.20 0.22±0.01	1.82±0.19 0.14±0.02
C	Holding time 0-30 min	+	1.95±0.10	1.63±0.13
D	Control	+	3.13±0.15	1.91±0.07

[1] See Table 4.9.

[2] See Table 4.10.

[3] See Table 4.11.

rate during the dry heat treatment due to insufficient moisture content of the seed. Low moisture, in addition, prevents inactivation of indigenous myrosinase. Results for treatment (A) where myrosinase was not added as well as for those where the enzyme was added suggest that, after heat treatment, myrosinase, not being inactivated, causes a partial hydrolysis of glucosinolates.

In the case of the steam treatment (B), addition of myrosinase gave concentrations for 3-BITC and 5-VOT that were closest to the control. The same treatment without addition of myrosinase resulted in the lowest levels both of 3-BITC and 5-VOT. Steam was much more efficient in releasing glucosinolates than was dry heat. The low level of autolysed glucosinolates in the meals from steam treated seeds can be attributed partly to a steam distillation of volatile isothiocyanates occurring under the conditions of circulating steam, but also to more efficient myrosinase inactivation in the presence of the higher moisture contents of the seeds, which correlates with the findings of Kozlowska *et al.* (1983) and Ochetim *et al.* (1980).

There was a discrepancy between the low concentration of autolyzed glucosinolates in the meal from the steamed seeds and the high concentration of sulfur in the oil from the same treatment. The explanation for this phenomenon could be in a possible nonenzymatic, thermal degradation of glucosinolates upon steam treatment of the seed, giving rise to lipid-soluble, sulfur-containing compounds. This is supported by earlier observations, summarized by Olsen

(1980), that glucosinolates are not very stable in aqueous solutions.

4.4 Bleaching of Commercial Oil with Natural and Acid-activated Clay

Graphs (Figures 4.14 and 4.15) illustrate the bleaching performance of three different adsorbents. Activated Na-neutralized clay (Tonsil brand) appeared to be the best agent, although the bleaching effect was noticable only at concentrations >500 ppm. Brazilian naturally-active clay was less effective Tonsil; chlorophyll was reduced from 20.8 ppm to 16.3 ppm with 1% added natural clay, while Tonsil, at the same concentration, caused a decrease in chlorophyll concentration from 21.1 ppm to 8.2 ppm. A similar pattern was observed in removal of carotenoids from the oil (Figure 4.15).

The green color in vegetable oils, due to chlorophyll, is much more responsive to a slightly acidic earth than to one of the ordinary type, because the pigment is unstable under acidic conditions (Norris, 1979). This probably explains the differences among the results of our experiment.

4.5 Suggestions for Future Research

At the maximum allowed holding time (30 min) in our experiments, no lipolytic activity was observed. This can be due to insufficient holding time and to the low moisture content of the seed. It would be of interest to evaluate

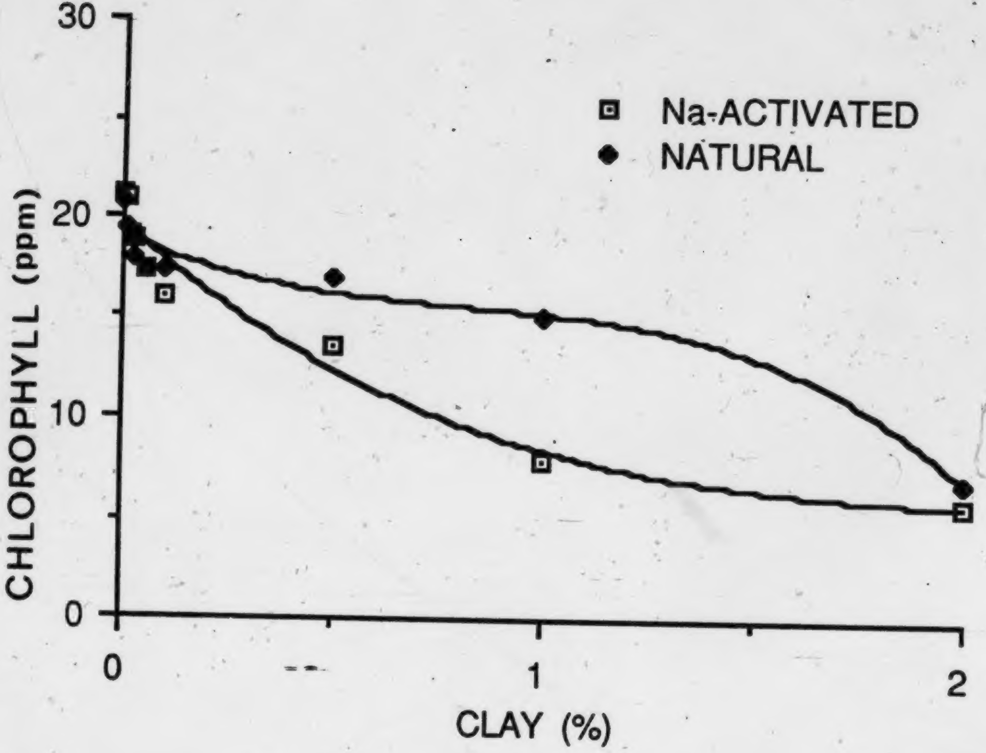


Figure 4.14 Efficiency of Na-activated and natural (Bentonite) clay on chlorophyll removal from commercial canola oil. Points on graph represent average of three replicates.

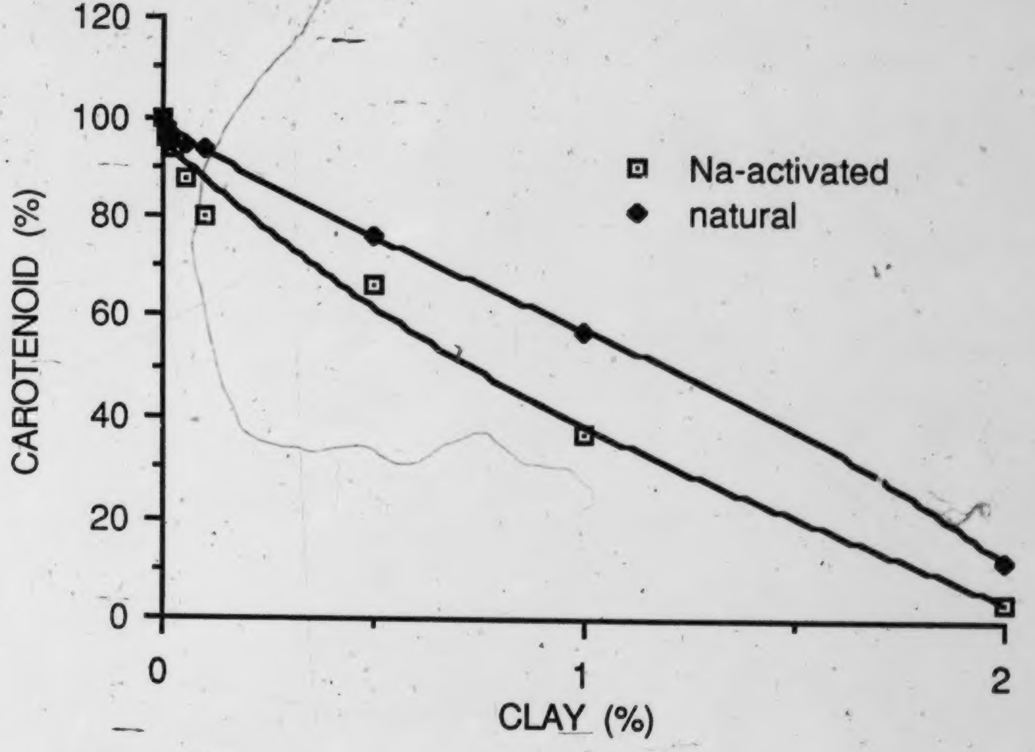


Figure 4.15 Efficiency of Na-activated and natural (Bentonite) clay on carotenoid removal from commercial canola oil. Points on graph represent average of three replicates.

this activity more precisely as a function of holding time and the moisture of the seeds.

Increased levels of phosphorus in the oil from steam-treated seeds certainly proved the ability of steam to enhance the yield of compounds with polar groups, such as phospholipids and glucosinolates, upon pressing and/or solvent extraction. Qualitative and quantitative analysis of phospholipids obtained from steam treatment would reveal information on the proportion of hydratable versus nonhydratable phosphatides, which is of interest to the processing industry. Furthermore, it would give valuable data on the relative composition of PC, PE and PI, particularly with respect to their different emulsifying properties.

Rapeseed phospholipids in general had not found their place on the emulsifier market mainly because of their inferior quality to soybean phospholipids and their uneconomical production.

The palatability problem that was partly associated with the high content of glucosinolates in earlier cultivars has been overcome through breeding. It appears, however, that steam treatment would further improve phospholipid quality through its efficient inactivation of myrosinase. Other organoleptic problems, associated with the formation of melanophosphatides, could also be monitored in the steam-treated samples.

Electron microscopic study of the effects of steam treatment as compared to the dry heating would reveal exactly how efficient steam is in rupturing oil-bearing cells and in facilitating lipid extraction. Treatment of seeds with solvents of differing polarity would enable location of the exact position of polar and nonpolar lipids in canola seed.

Increased sulfur and chlorophyll concentrations in the oil are definitely among the strongest "contras" against steam treatment. Therefore, additional experimentation would be required in order to find which combination of steaming time, postreatment time and temperature would yield an oil of optimum composition.

From the point of view of glucosinolate content in the meal, steam treatment would probably be a suitable replacement for the traditional process because it eliminates the need to control the minimum moisture in the seed and the content of autolyzed glucosinolates in the meal becomes negligible.

However, since the amount of intact glucosinolates in the meal increases, qualitative and quantitative analysis would present more information on their status in the seed. The HPLC method used by Olsen and Sorensen (1980) would be most appropriate due to its nondestructiveness of the samples.

Furthermore, nitriles produced from glucosinolate hydrolysis are not detectable by the thiourea-UV method. Toxicity of nitriles in very low concentrations, coupled with the fact that myrosinase at low seed moistures resists inactivation, would require the use of different methods (e.g. gas chromatography) for nitrile quantification, as would a comparison between dry heat and steam treatments.

One of the most valuable contributions to this work would be the analysis of canola meal protein as affected by steam treatment. The favorable amino acid pattern in canola meal allows its use as a supplement to soybean meal in animal feed. How steam treatment would alter canola protein and its amino acids (particularly lysine and methionine) remains to be analysed.

5. CONCLUSION

Lipase and lipoxygenase activities were not evident in seed exposed to 0-30 min holding time at room temperature. The composition of lipid fractions and of their fatty acids did not vary significantly with the duration of holding time. Yield of neutral lipids (triacylglycerols) as well as the fatty acid pattern in all lipid fractions did not vary significantly between the dry heat and steam treated seed. There was a highly significant increase in concentrations of phosphorus, sulfur, chlorophyll and carotenoids in both prepressed and solvent extracted oils from steam treated seed. Extended steaming time was found to cause a further increase in phosphorus content, while the same treatment, combined with higher temperature of drying, resulted in oil with significantly higher sulfur concentration.

There was no significant correlation between the time of dry heat or steam treatment of the seed and the corresponding concentrations of 3-butenylisothiocyanate and 5-vinyloxazolidinethione in the meal. However, meals from steam treated seed were on average significantly lower in autolyzed glucosinolates, but at the same time significantly higher in total glucosinolates, suggesting that steam was more efficient than dry heat in myrosinase inactivation and also in releasing glucosinolates from their bound form.

Application of steam treatment instead of conventional dry cooking would probably result in oil with increased concentration of phosphorus, sulfur, chlorophyll and carotenoids, which would require higher refining and bleaching costs. This would be partly compensated for through the improved palatibility and nutritional quality of the meal, and through seeking ways of making canola lecithin a marketable product. While final conclusions can be drawn only after a detailed feasibility study, results of this study suggest that, at present, steam treatment of canola seeds would not be an appropriate alternative to the traditional process.

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